CYCLODEXTRIN INCLUSION COMPLEXES FOR IMPROVED DRUG ACTIVITY

Department of Chemistry
Shailabala Women's Autonomous College, Cuttack

SWAPNA SANKAR NAYAK PhD

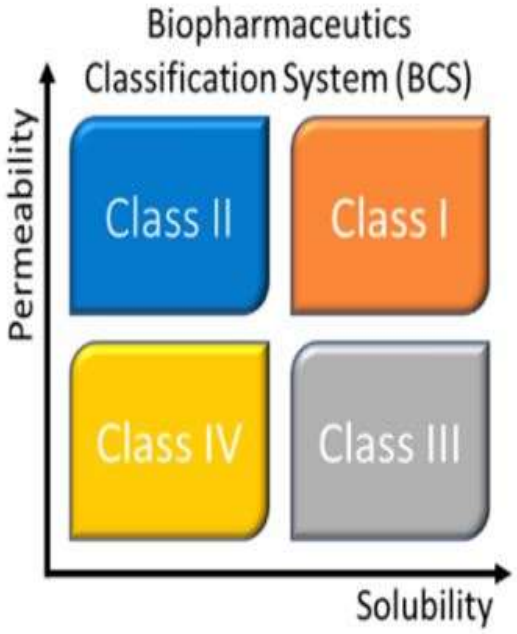
"How will that stuff get from down there up to my sore throat?"

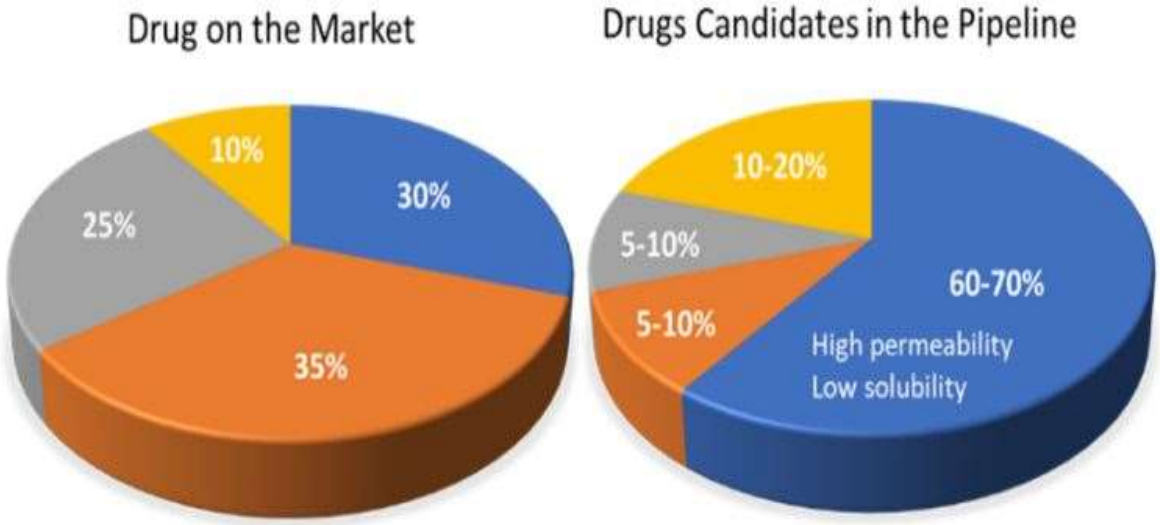

Nimesulide Tablets 100 mg
Namosid
(Analgesic and antipyretic Tablets)
Osteoarthritis, Pain of malignancy, post-operative, trauma, sports injuries, ENT, dental & gynecological disorders.
5 x 10 x 10 Tablets
100 mg.


Cynim-MD
Nimesulide
(In Beta-Cyclodextrin)
Dispersible Tablets
Cynim-MD
20 x 10 Tablets


ORGAPROX
1 x 10 Tablets
Rx
Piroxicam Capsules USP
ORGAPROX
GMP
C&C ORGANICS


20 scored tablets
(Hoover Specs.)
Roxibeta
(Piroxicam Beta-Cyclodextrin)
Generic Name:
Piroxicam
Beta-Cyclodextrin
Strength:
20 mg
Pack Size:
2x10 Tablets
SERVING WITH ALL SINCERITY AND DEDICATIONS





Class 1
High solubility
High permeability

Class 2
Low solubility
High permeability

Cyclodextrin complexation

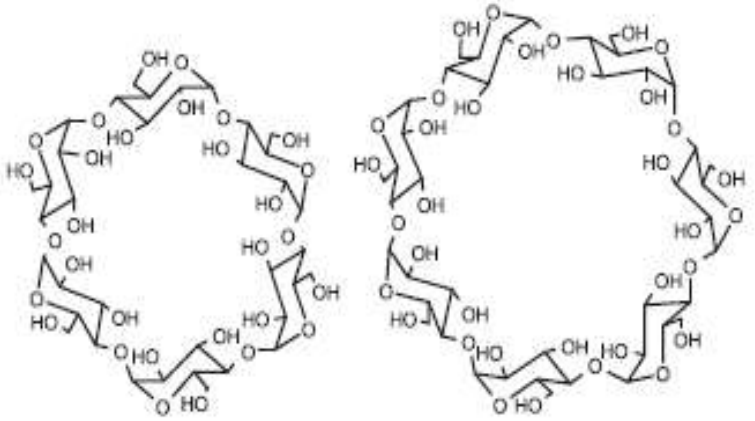
Class 3
High solubility
Low permeability

Class 4
Low solubility
Low permeability

Cyclodextrins

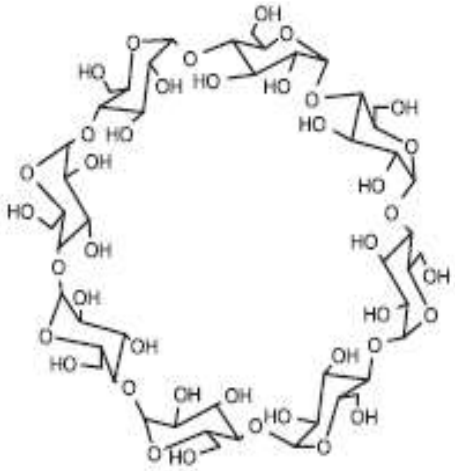
Molecules with cavities...

Stoddart: ‘‘*Cyclodextrins are all-purpose molecular containers for organic, inorganic, organometallic, and metalloorganic compounds that may be neutral, cationic, anionic, or even radical*”.



α β

glucopyranoside units



γ

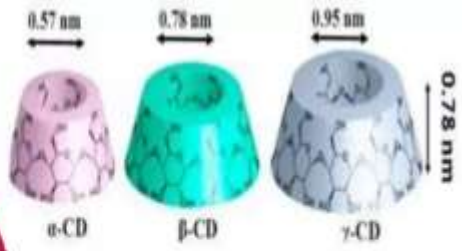
Cyclodextrins comprise of a family of cyclic oligosaccharides and are topologically represented as toroidal macro rings built up from glucopyranose units

α-CD: Cyclohexamylose comprises six glucopyranose units

β-CD: Cycloheptaamylose comprises seven glucopyranose units

γ-CD: Cyclooctaamylose comprises eight glucopyranose units

Three forms of Cyclodextrins available



Alpha(ą)

- Used Parenterally.
- Used with small drug molecules due to very small cavity.

Beta(ß)

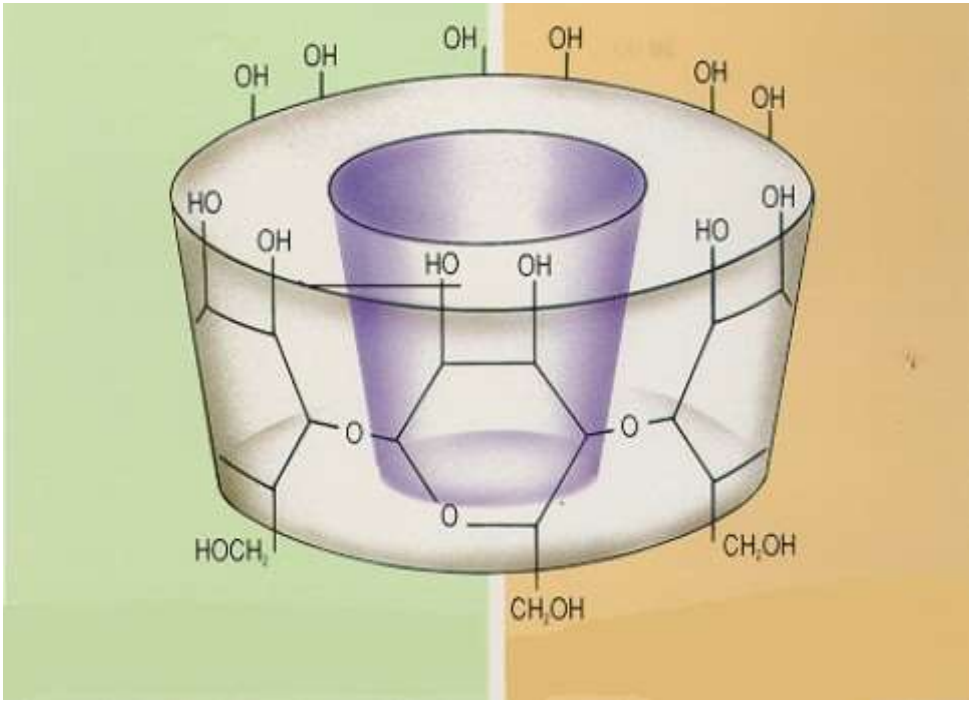
- This form mainly used.
- Least soluble but least expensive.
- Commercially available from number of sources.
- Compatible with many drugs and molecules.
- Nephrotoxic when given parenterally.
- Whereas nontoxic when given orally through tablets and capsules
- Can be wet granulated as well as direct compression

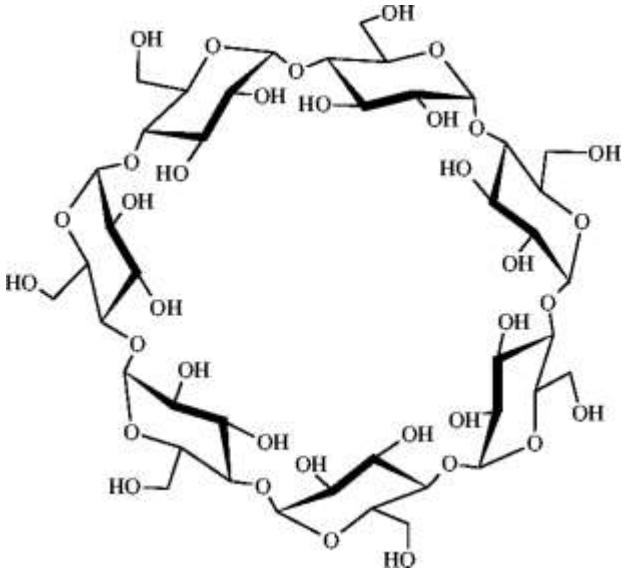
Gamma(Ý)

- This form has largest cavity
- Low toxicity
- Enhanced solubility

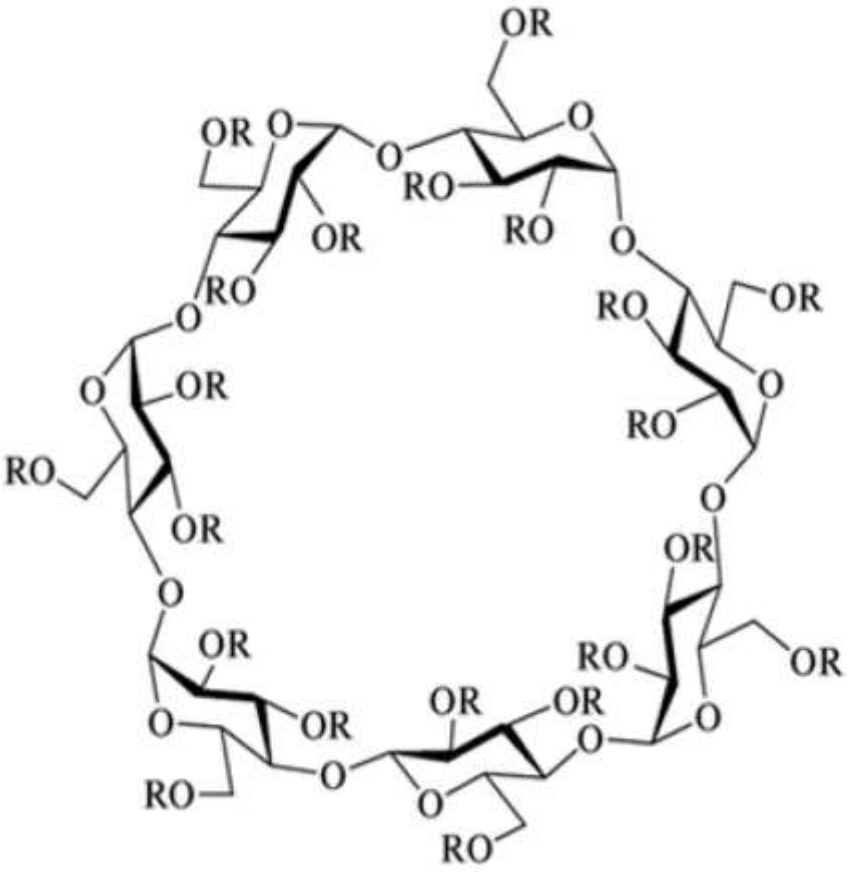
STRUCTURAL FEATURES

Both types of hydroxyl groups are situated at **the exterior** separately on either edges of the cone (torus) -CH_2OH groups at the narrow edge and >CHOH groups at the wider edge. **The interior** of the torus consists only a lining of hydrogen atoms and glycosidic oxygen bridges. The interior of the toroids is **hydrophobic** while the exterior is sufficiently **hydrophilic** to make CDs water soluble.





DERIVATIVES OF β-CYCLODEXTRIN

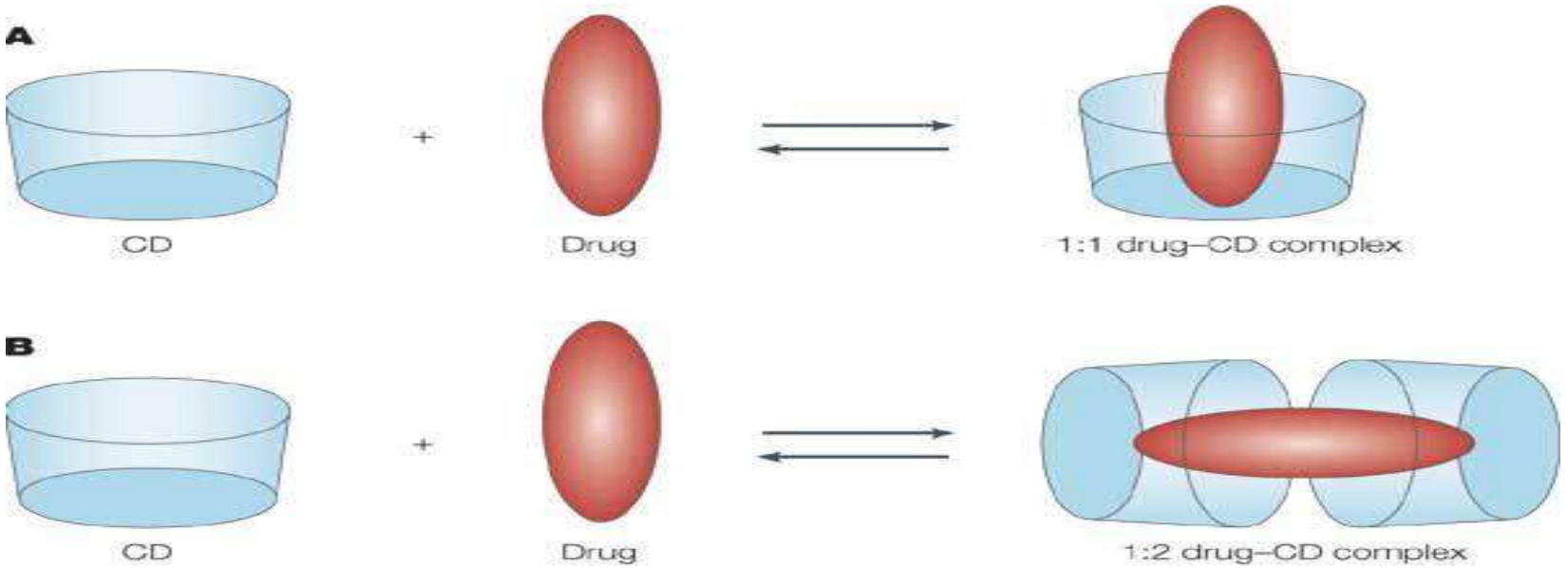


Cyclodextrin	R =
MβCD	$-CH_3$
HPβCD	$-CH_2CH(OH)CH_3$
CMβCD	$-CH_2COONa$
SBEβCD	$-(CH_2)_4SO_3Na$

CD Inclusion Complex Formation

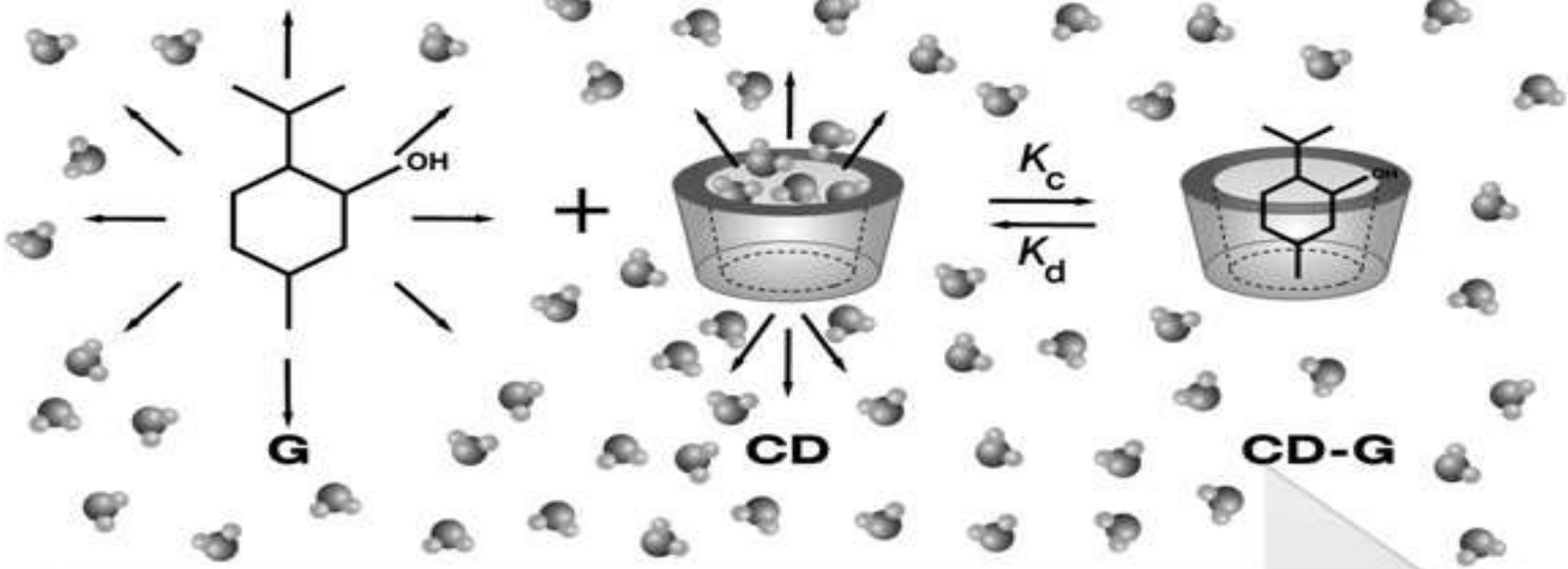
Inclusion complex is formed when a hydrophobic "guest" gets entrapped in the cavity of "host" CD, a process with dynamic equilibrium driven by *Loss of free-energy & Gain in entropy*

- minimum requirement for inclusion complex formation is a size compatibility between host and guest.



MECHANISM

$$K_c = K_{1:1} = \frac{[CD\text{-}G]}{[G]\,[CD]} \qquad K_d = \frac{1}{K_c}$$

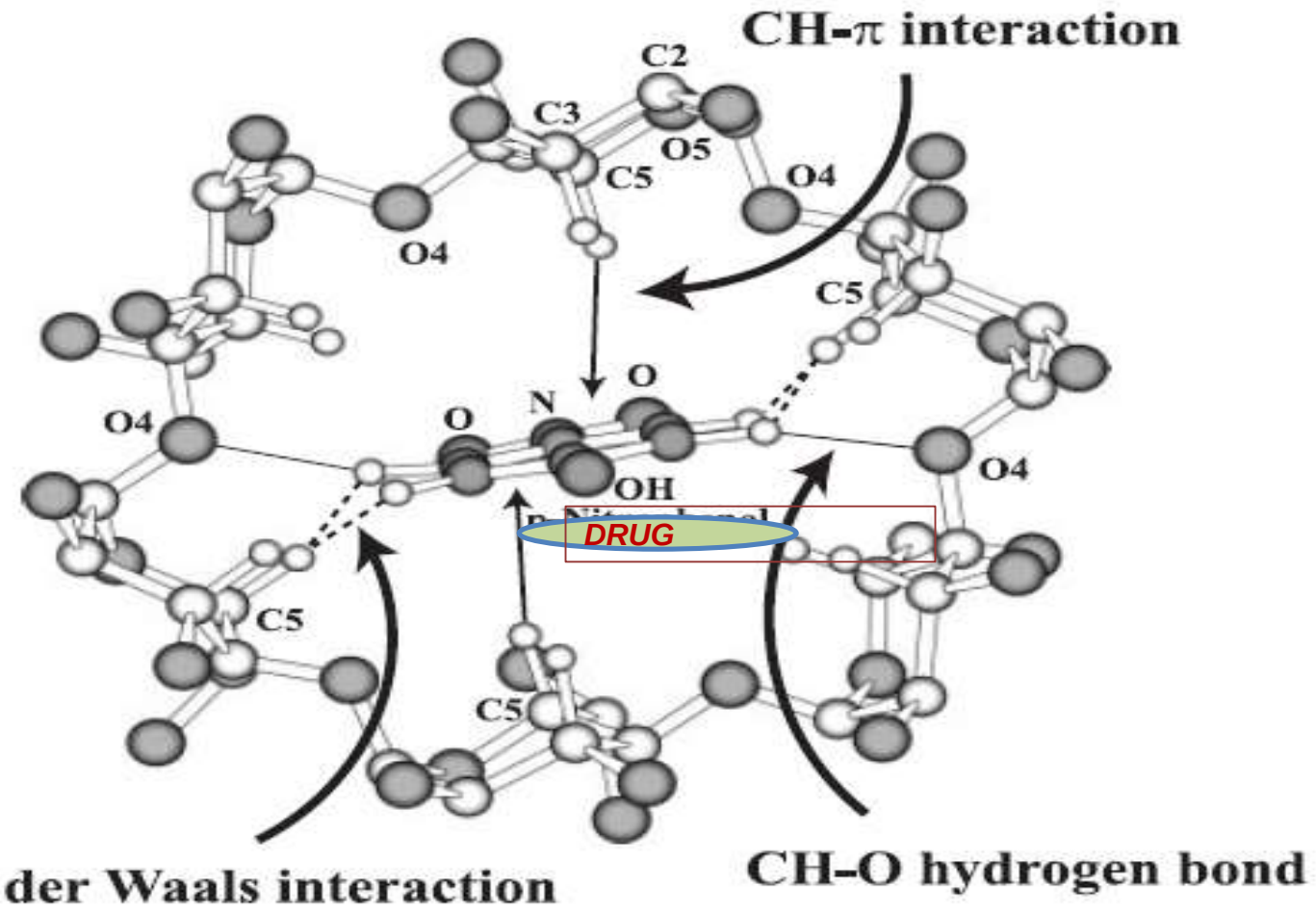


A reduction of the repulsive interactions between the hydrophobic guest and the aqueous environment.

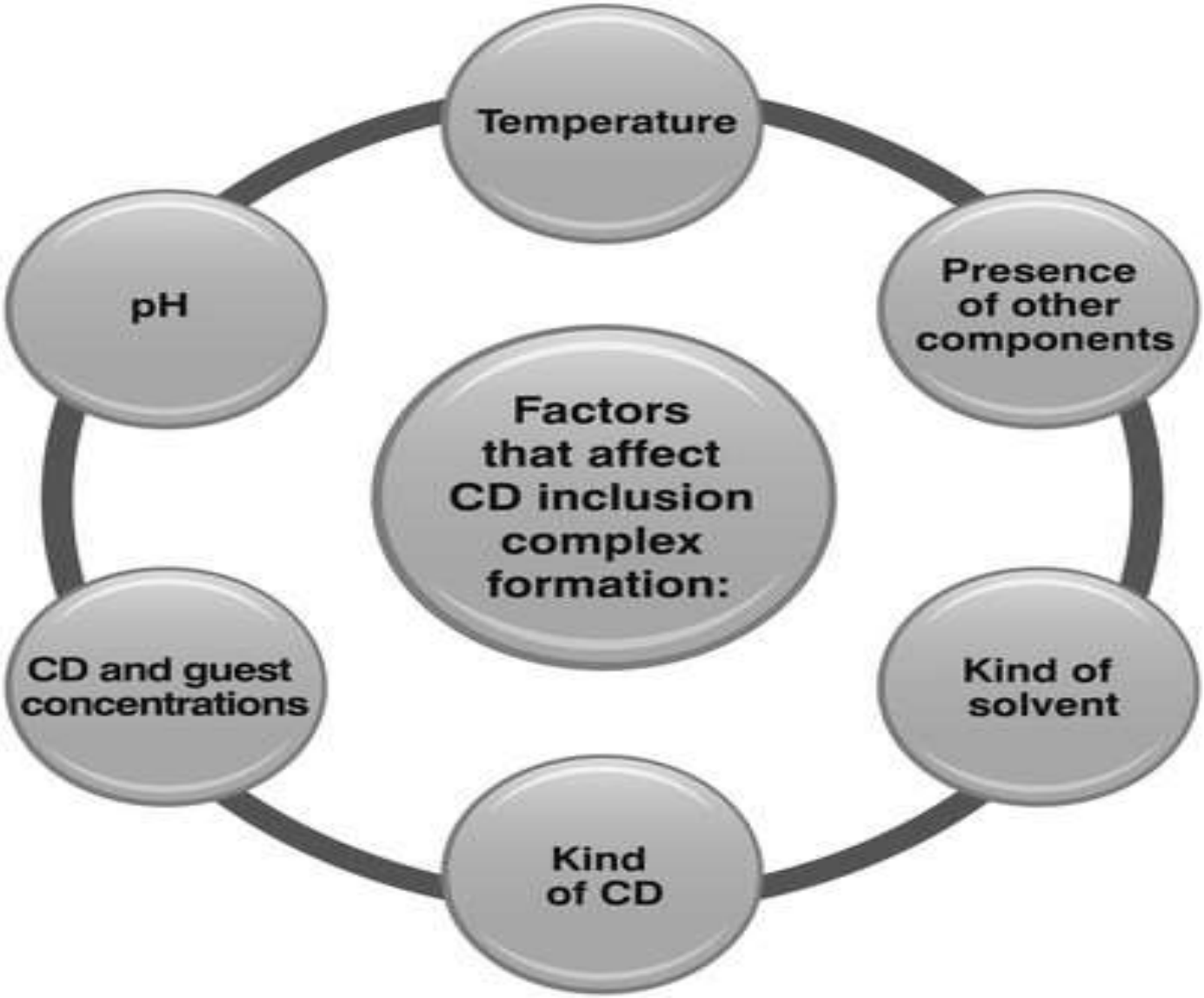
The displacement of polar water molecules from the apolar cyclodextrin cavity.

An increase in the hydrophobic interactions as the guest inserts itself into the apolar cyclodextrin cavity.

Host–Guest Interactions



Non-covalent interactions like hydrophobic interaction, vander Waals forces and hydrogen bonds lead to the formation of inclusion complexes.

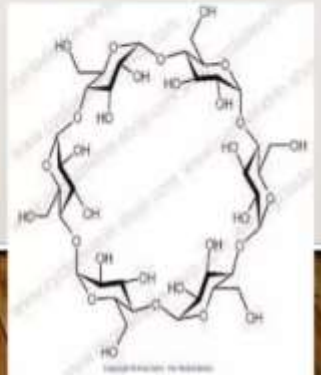

Temperature
Presence of other components
Kind of solvent
Kind of CD
CD and guest concentrations
pH
Factors that affect CD inclusion complex formation:

Cyclodextrins are Crystalline, non hygroscopic, cyclic oligosaccharides derived from starch

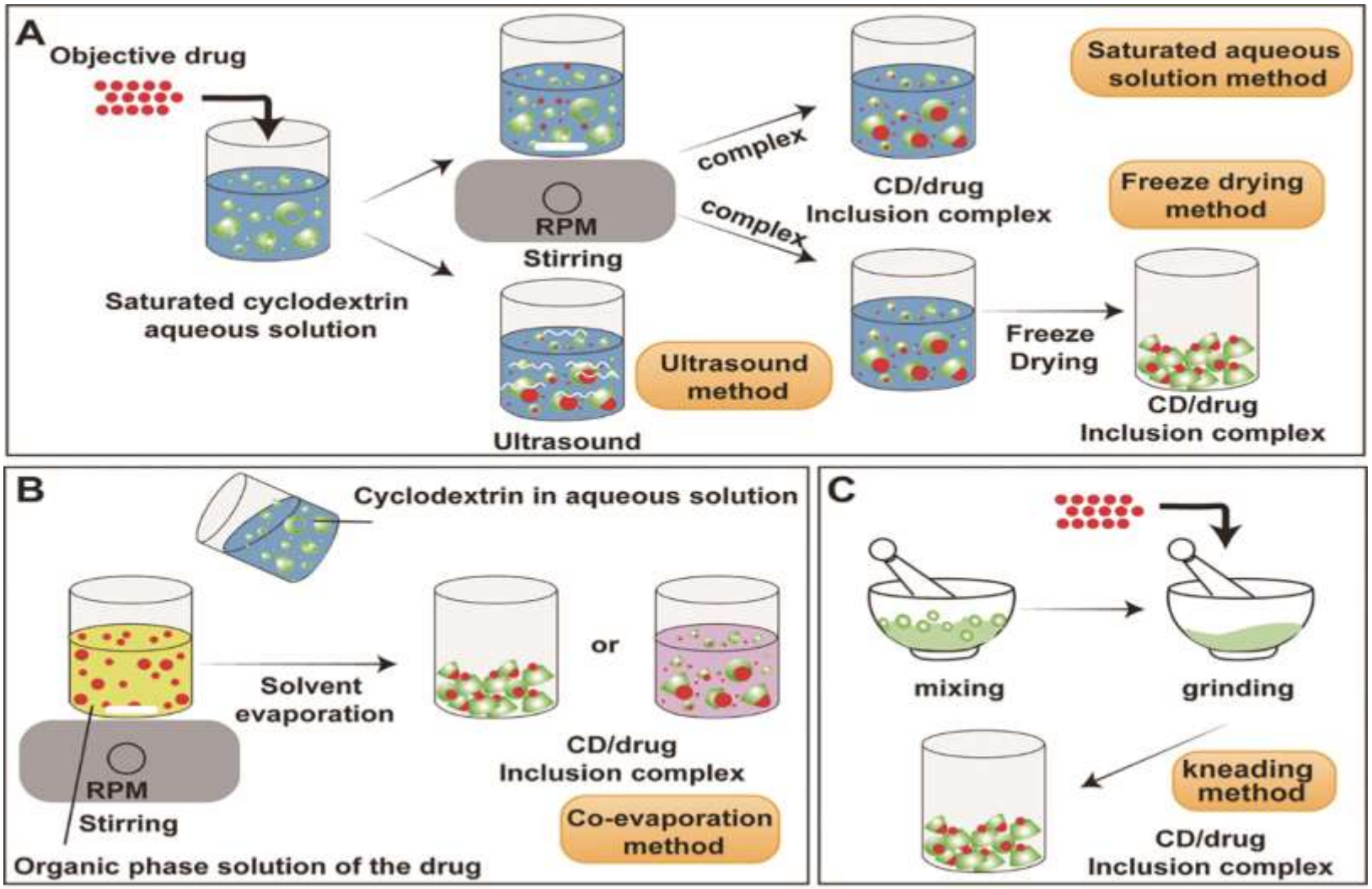
Cyclodextrins are inclusion compounds

- Hydrophilic Exterior surface and nonpolar interior cavity.
- Forms noncovalent bonds with hydrophobic drugs
- Entraps single guest molecule in cavity of host molecule

- Due to arrangements of molecules and hydroxyl groups



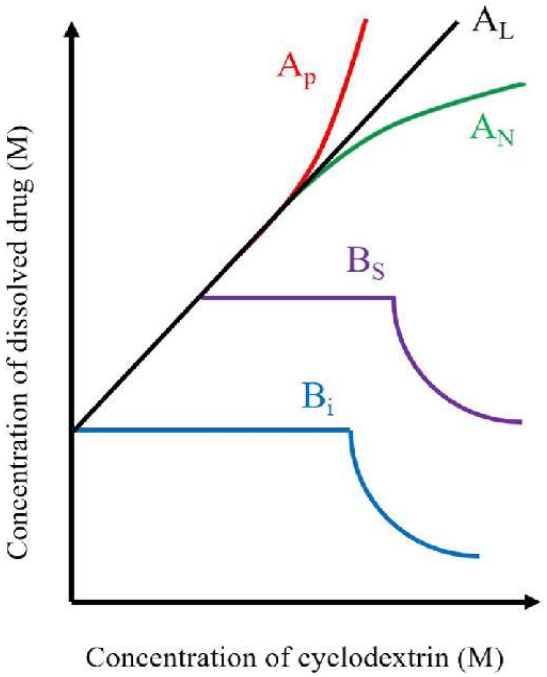
PREPARATION OF β-CD INCLUSION COMPLEXES

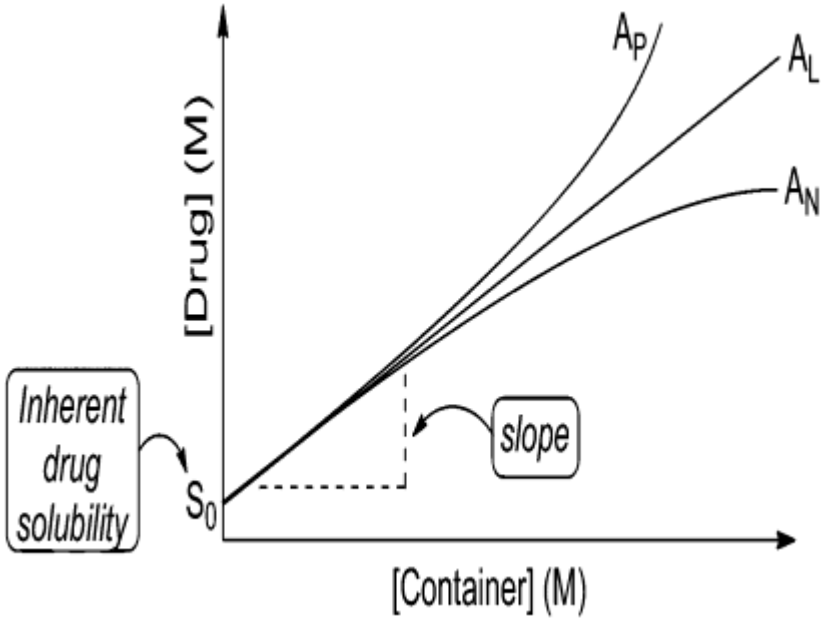


- **Phase Solubility Studies**
- **Higuchi and Connors method-phase solubility curves-to ascertain the stoichiometry- To find stability constant K**

Phase-solubiliy fall in two main categories, A-type curves are indicated for the formation of soluble inclusion complexes while B-type behaviour are suggestive of the formation of inclusion complexes of poor solubility. The A-curves are subdivided into A_L (linear increases of drug solubility as a function of cyclodextrin concentration), Ap (positively deviating isotherm) and A_N (negatively deviating isotherms) subtypes.

$$K_s = \frac{\text{slope}}{S_0\,(1 - \text{slope})}$$





Experimental Methods

- **Thermodynamic Studies**
- To determine ΔG, ΔH & ΔS for the inclusion complexation from the temperature dependency of the binding constants of the complexes
- Curve with linear regression obtained by plotting ln OD vs. 1/T which are fitted to van't Hoff eqn:

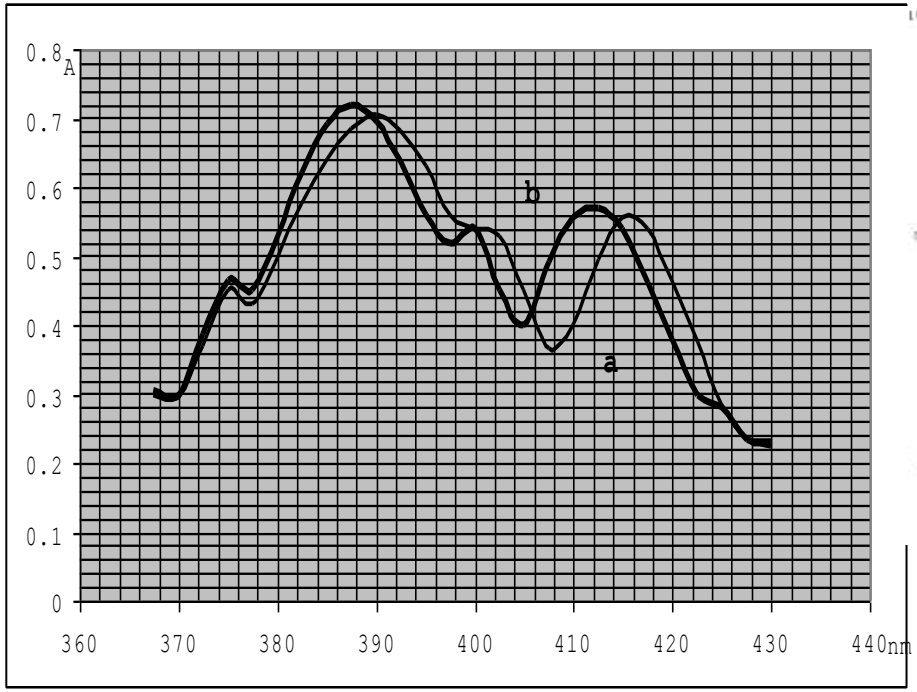
$$\ell n\ K = \left(\frac{-\Delta H}{RT}\right) + \frac{\Delta S}{R}$$

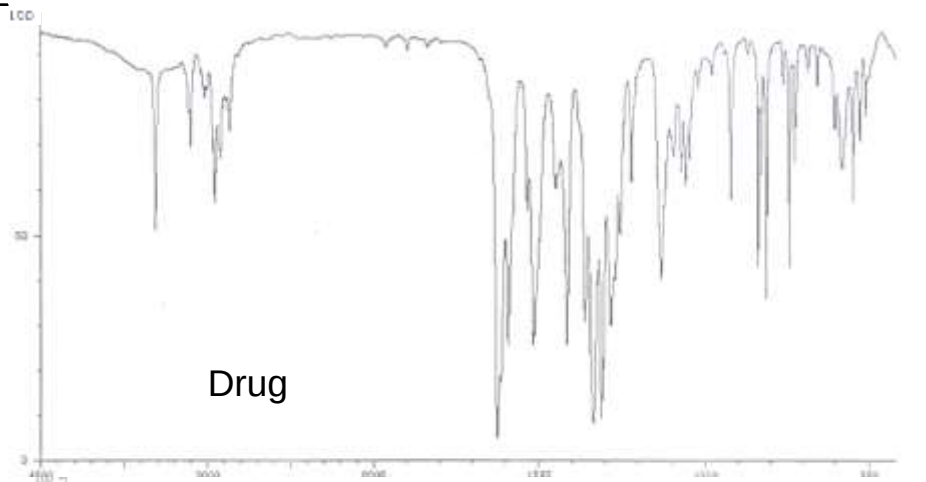
- The slope of the plot corresponds to $\frac{-\Delta H}{R}$. The ΔG and ΔS values were obtained using the following equations
- $$\Delta G = -RT\ \ell n\ K$$
- $$\Delta G = \Delta H - T\Delta S$$

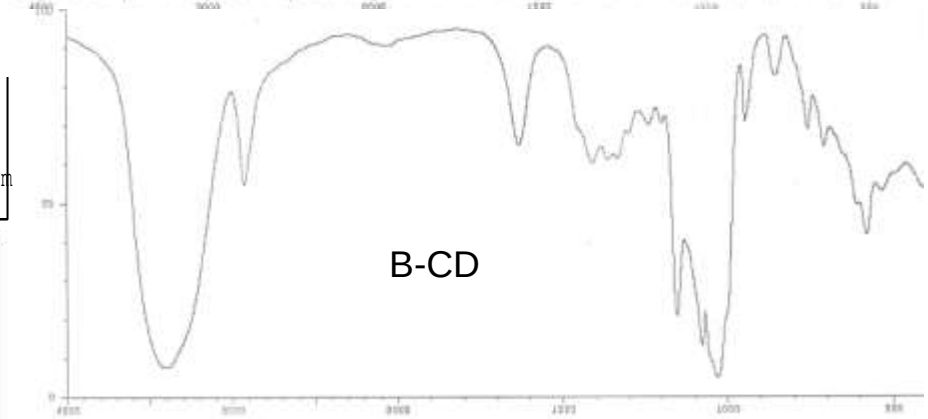
Study of Host-guest interaction in complex formation

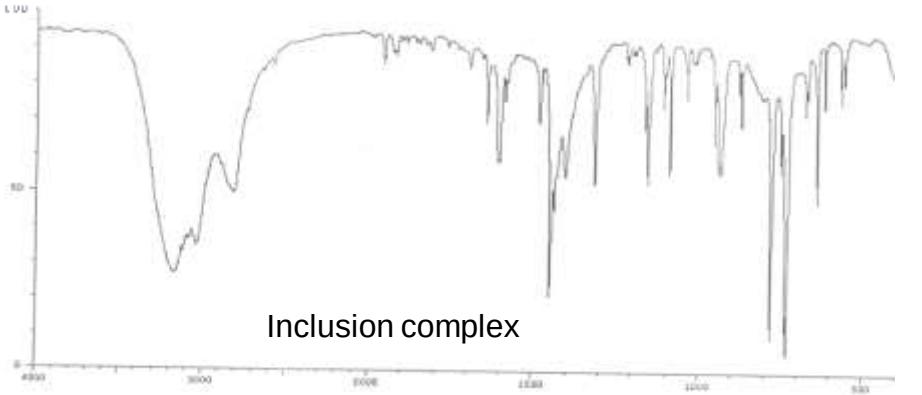
* A higher shift in the melting point indicated that these compounds were encapsulated in the β-CD cavity.
* Both hypsochromic and hyperchromic shift to a small extent which might be due to a change in environment (polarity) of the drug found during complex formation.
* Some characteristic IR peaks disappeared and new peaks appeared confirming the formation of inclusion complexes between drug and its derivative with β-CD.
* These molecules were incorporated within the hydrophobic cavity of β-CD and hence the peaks in the donor's IR spectra got broader and weaker.
* The interaction of donor (guest) and accepter (host) was based on the main binding forces including hydrogen bonding, vander Waal's forces and hydrophobic interactions. Since there was no large shifting of signals, the possibility of co-valent bonding in the inclusion complexes was excluded.

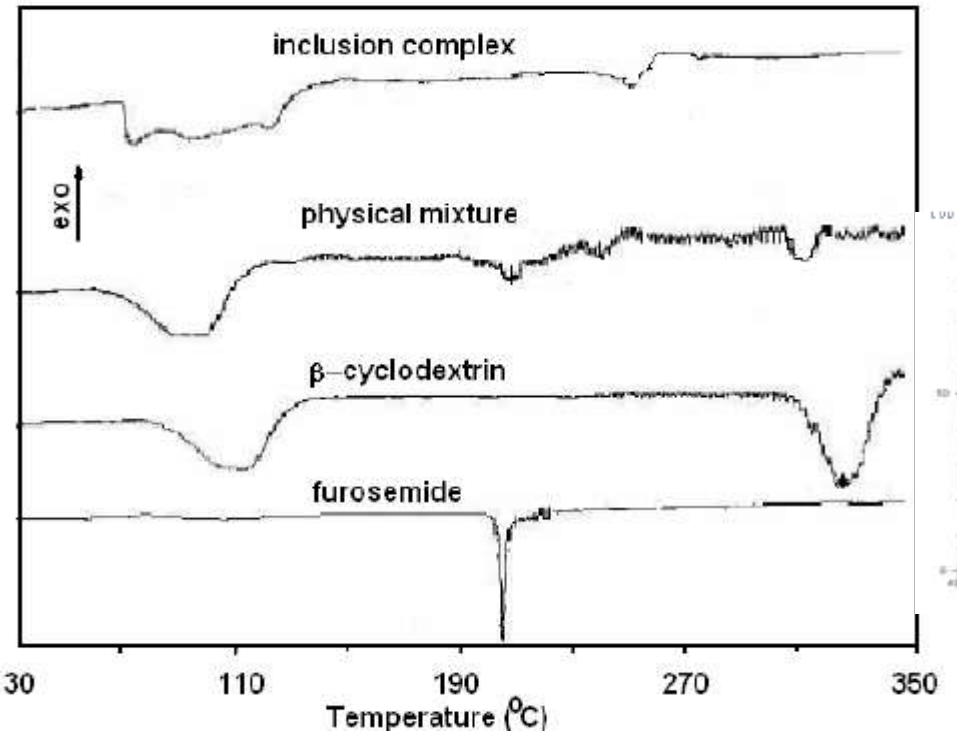
- The broad peak of β-CD at 3398 cm-1 had reduced its intensity in each spectra of the inclusion complexes indicating their formation
- The DSC curves of the simple mixture resemble the sum of the cure of two pure substances. After melting, a small exothermic peak is recorded, suggesting complex formation.


A
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
b
a
360
370
380
390
400
410
420
430
440nm


Drug

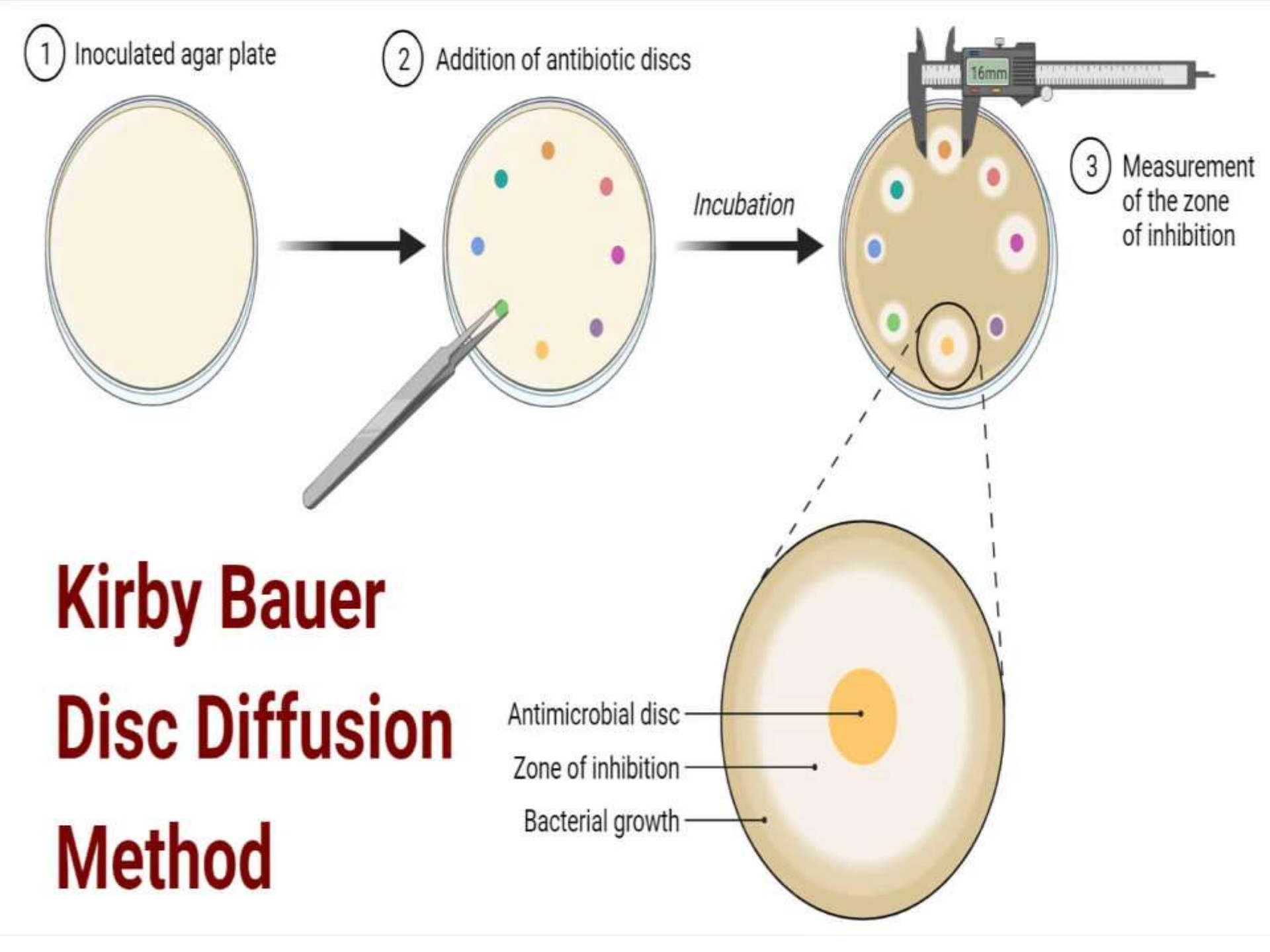

B-CD


Inclusion complex


inclusion complex
exo
physical mixture
β–cyclodextrin
furosemide
30
110
190
270
350
Temperature (°C)

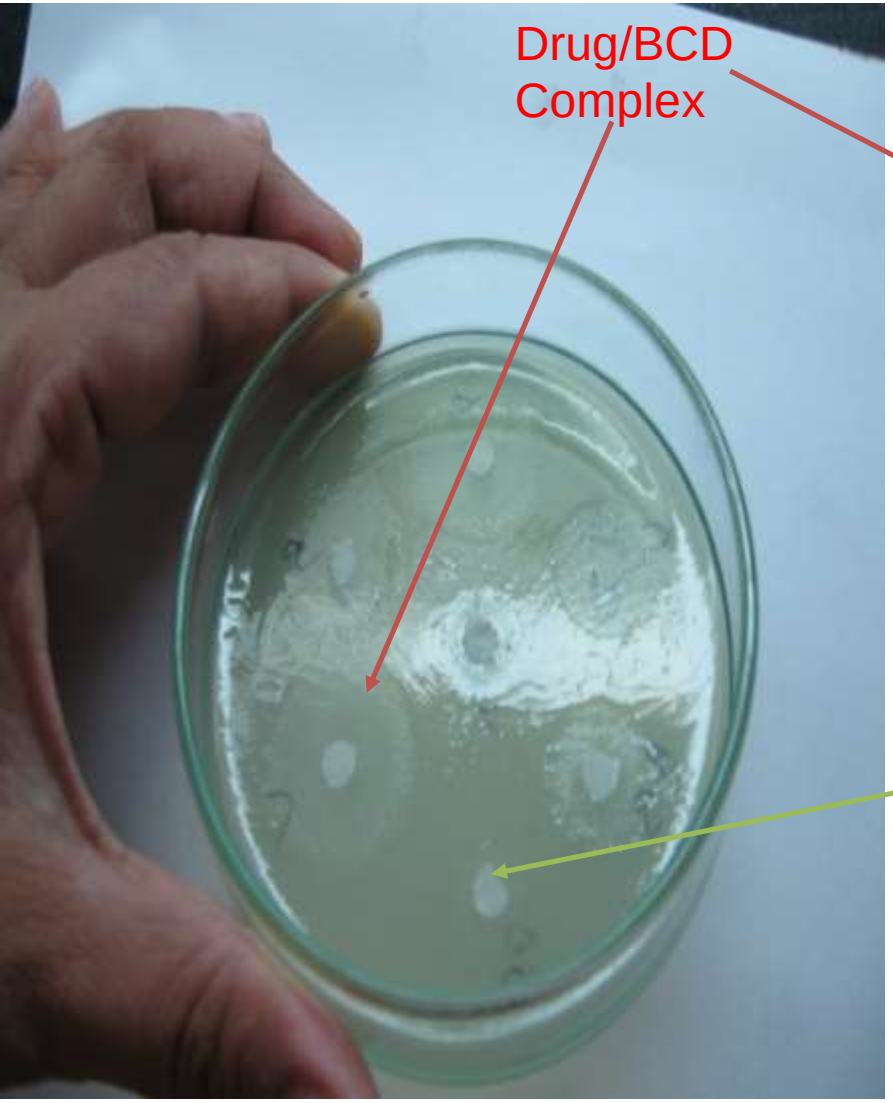
CYCLODEXTRIN EFFECTS ON IMPORTANT DRUG PROPERTIES

- Inclusion Complexation
- Solubility and dissolution enhancement
- Enhanced drug bioavailability
- Easier drug permeability
- Enhanced drug stability
- Improved drug safety
- Reduce gastrointestinal irritation, unpleasant smells/ tastes

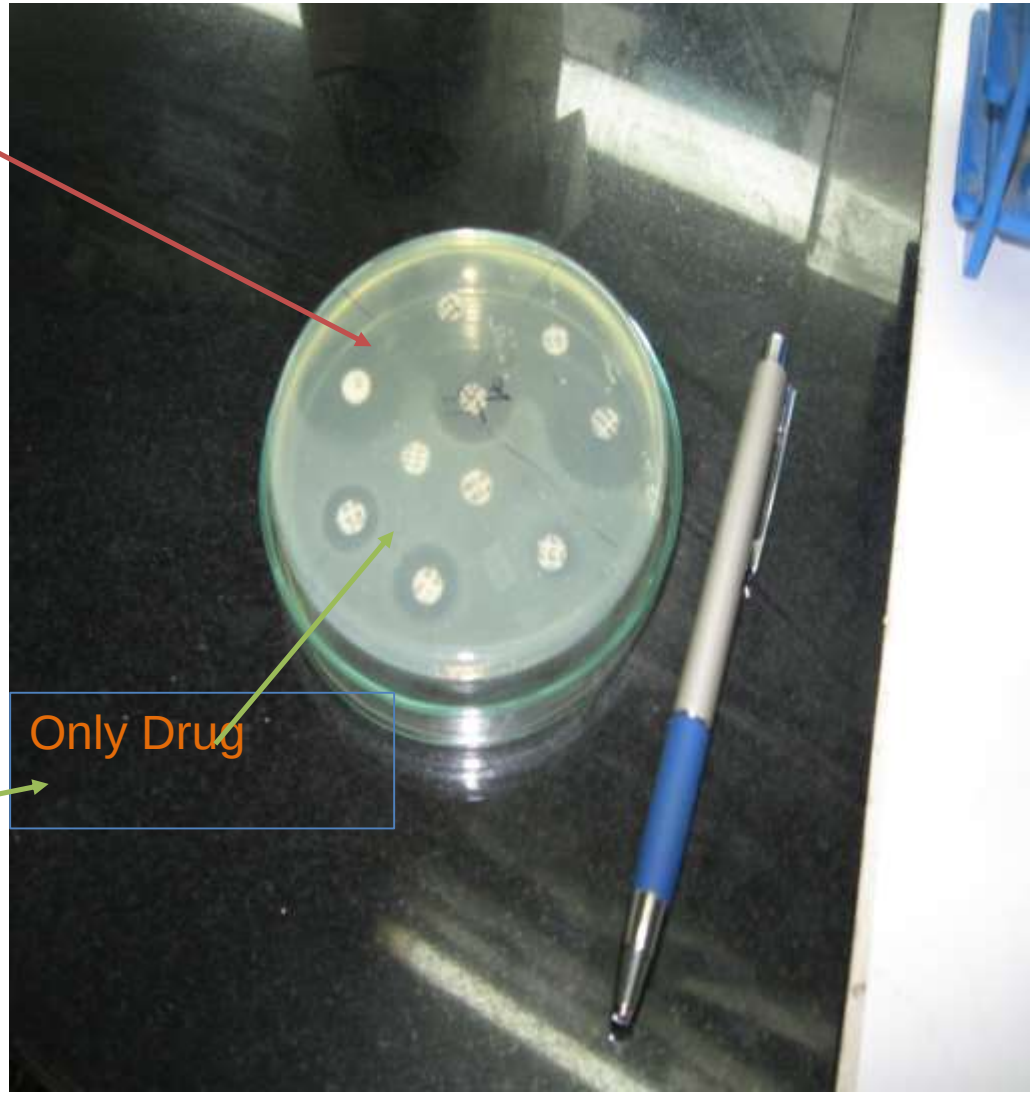


Kirby Bauer Disc Diffusion Method

Antimicrobial Study (Measurement of ZOI)



Disc diffusion test for *P. aeruginosa*



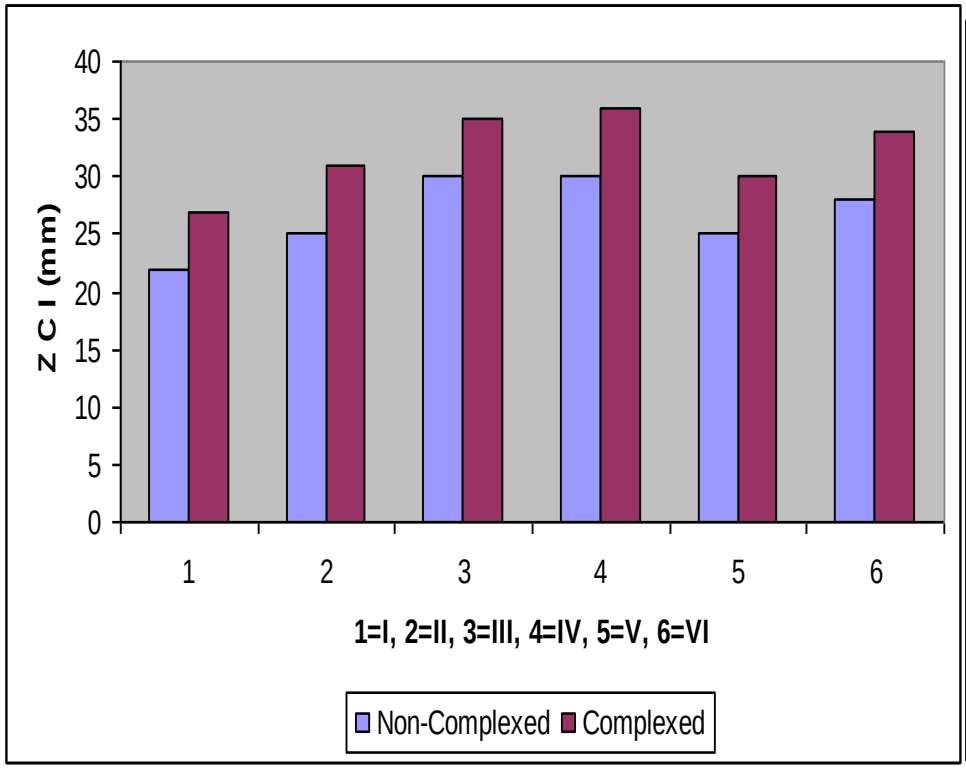
Disc diffusion test for *E. coli*

Antibacterial Sensitivity Reading

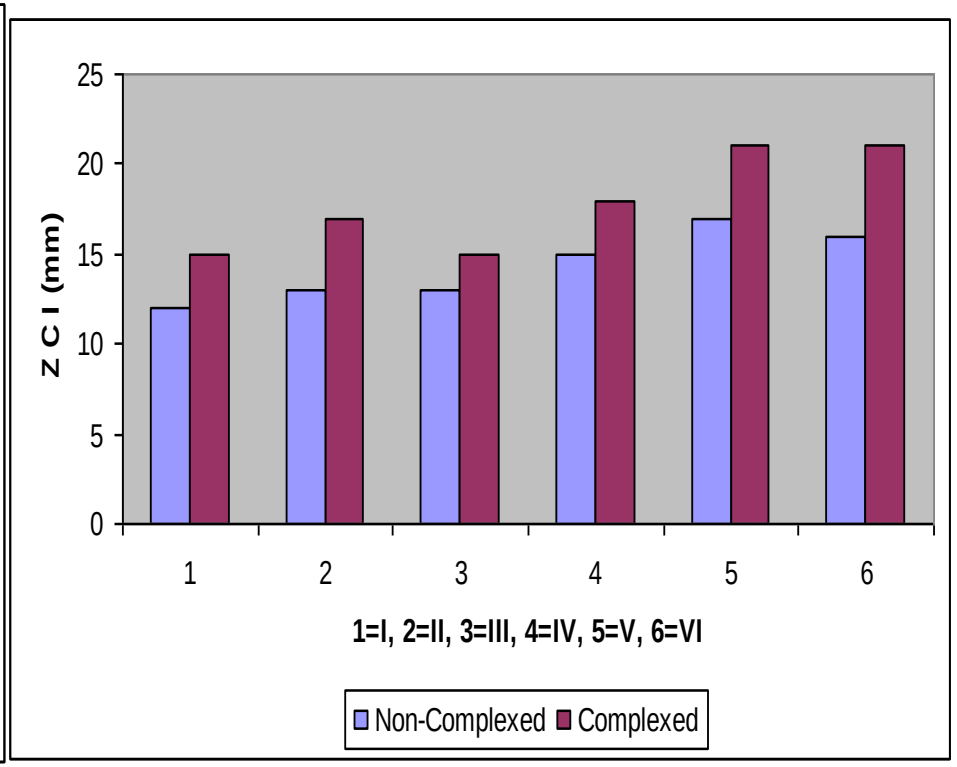
Sl. No.	Compound	Zone of Complete Inhibition (ZOI) in mm		
		S. Aureus	*P. Aeruginosa*	*E. Coli*
1	V	5(R)	24(S)	22(S)
2	Vc	8(R)	32(S)	30(S)
3	VI	5(R)	26(S)	26(S)
4	VIc	7(R)	38(S)	35(S)
5	Ciprofloxacin	30(S)	35(S)	35(S)

The antibacterial activity against *P. aeruginosa* of compounds V and VI had been increased by 20% and 25% while the same against *E. Coli* were 23.5% and 31.2% respectively after inclusion complexation.

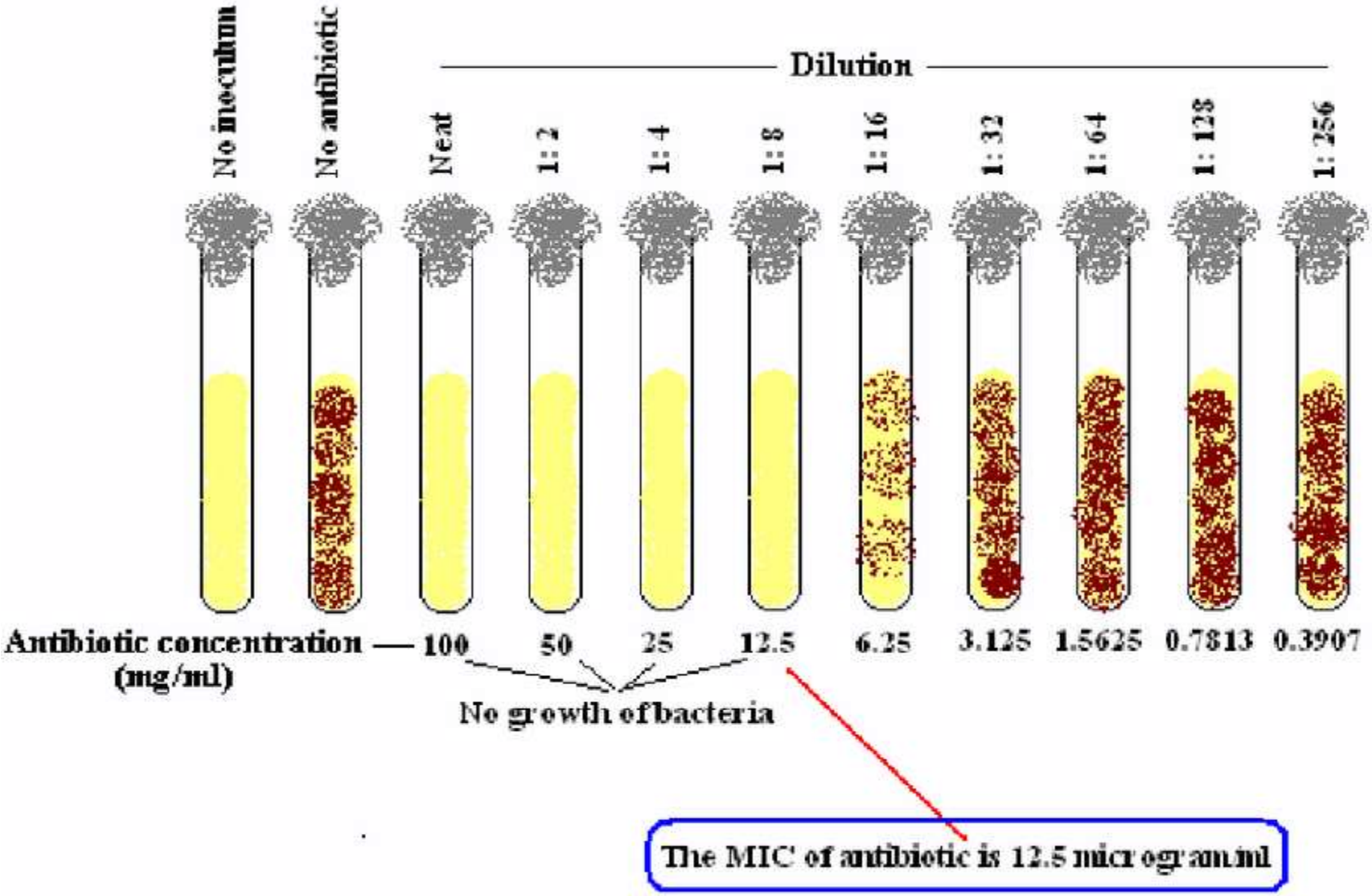
Z C I

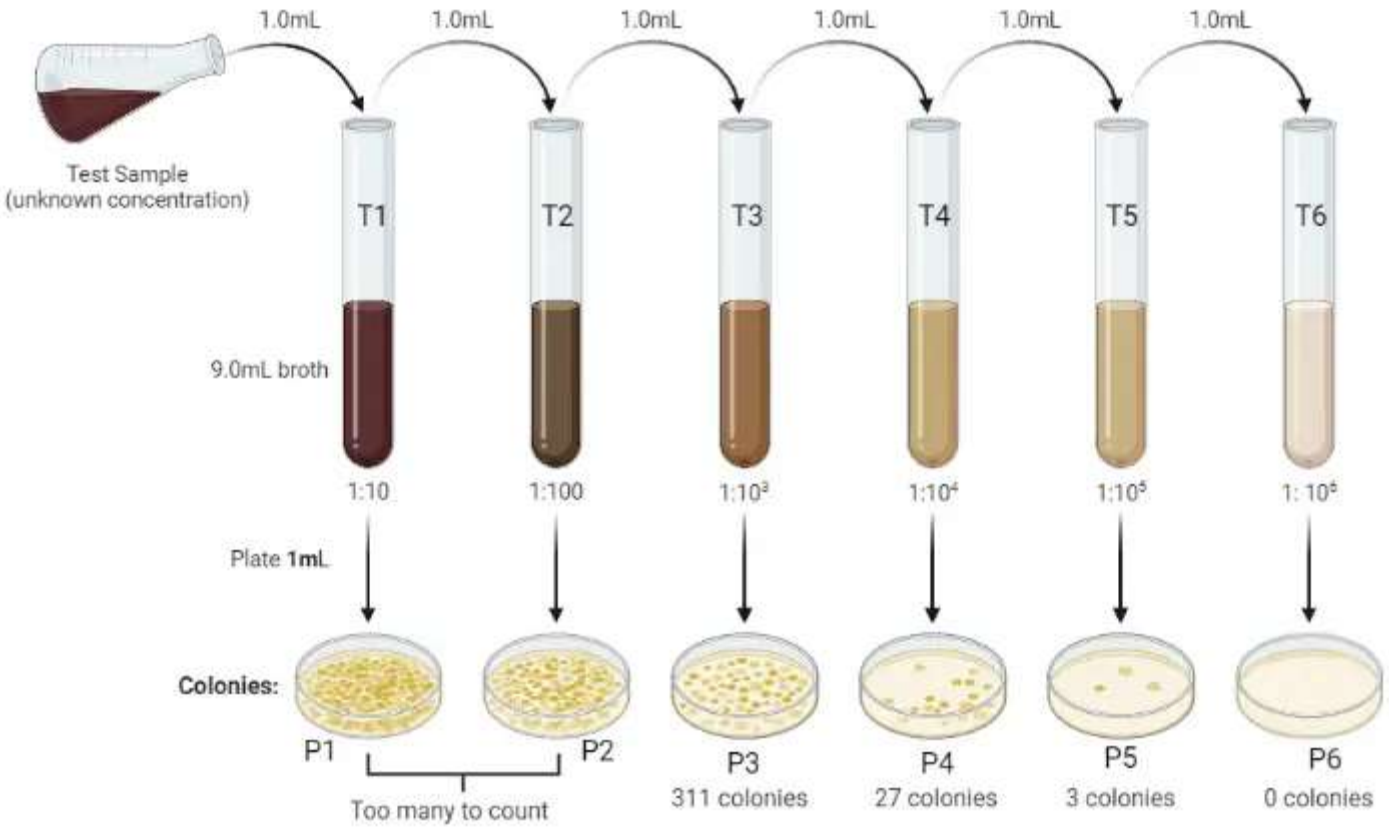


Z O I by acridone and derivatives (I-VI) against ***P. aeruginosa***



Z O I by acridone and derivatives (I-VI) against ***E. coli***


No inoculum
No antibiotic
Dilution
Neat
1: 2
1: 4
1: 8
1: 16
1: 32
1: 64
1: 128
1: 256
Antibiotic concentration (mg/ml)
100
50
25
12.5
6.25
3.125
1.5625
0.7813
0.3907
No growth of bacteria
The MIC of antibiotic is 12.5 micrograam/ml



CFU/mL = Number of colonies on plate x reciprocal of dilution of sample = number of bacteria/mL

311 colonies x 10^3 = 3.11 x 10^5 CFU/mL in sample

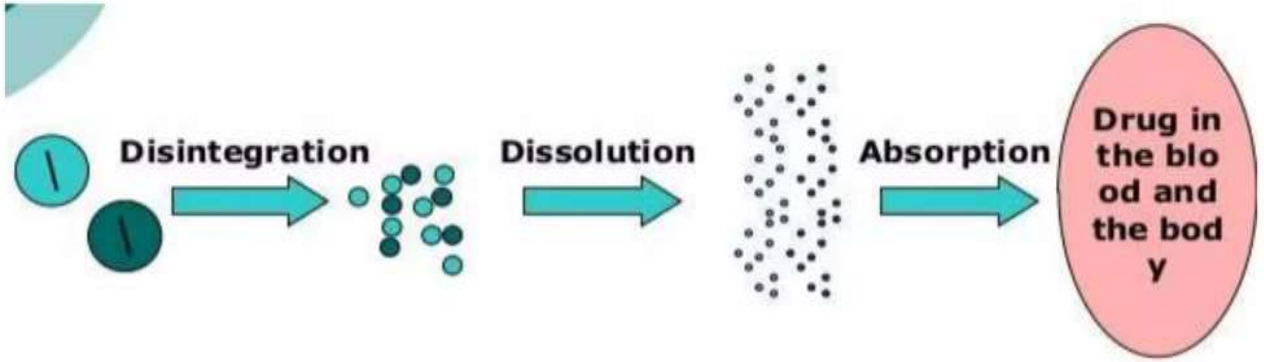
Determination of MIC/MBC (μg/ml)

Sl. No.	Compound	*P. Aeruginosa*		*E. Coli*	
		M I C	**M B C**	**M I C**	**M B C**
1	V	30	30	50	75
2	Vc	22.5	25.5	25	50
3	VI	35	40	50	75
4	VIc	27.5	30	50	50
5	Ciprofloxacin	22.5	25	25	25

What's the correct dose ?

DISSOLUTION :

- Dissolution is a process in which a solid substance solubilizes in a given solvent (mass transfer from the solid surface to the liquid phase.)
- Dissolution testing measures the extent and rate of solution formation from a dosage form, such as tablet, capsule, ointment, etc.
- The dissolution of a drug is important for its bioavailability and therapeutic effectiveness.



IN-Vitro Dissolution Study

- **Drug dissolution: A process in which the drug gets freed from its pharmaceutical form into solution in standard condition it can be absorbed by the organisms.**

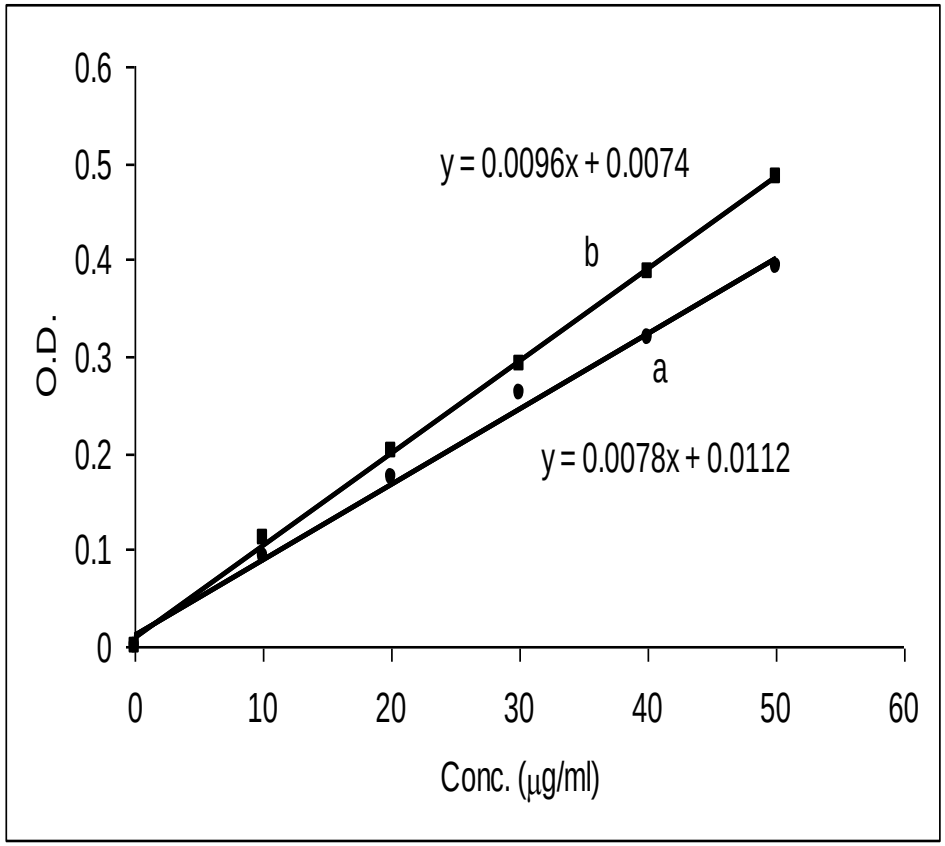
- **The *in-vitro* dissolution study requires the experimental set up similar to that of the *in-vivo* i;e a) same pH, same fluid volume of stomach, temp. and stirring speed etc.**

$$\underset{\text{complex (solid)}}{Drug\text{-}CD} \xrightarrow{K_d} \underset{\text{complex (solution)}}{Drug\text{-}CD} \underset{}{\overset{Kc}{\rightleftharpoons}} \underset{\text{free}}{Drug + CD} \xrightarrow{K_a} \text{drug absorbed in membrane.}$$

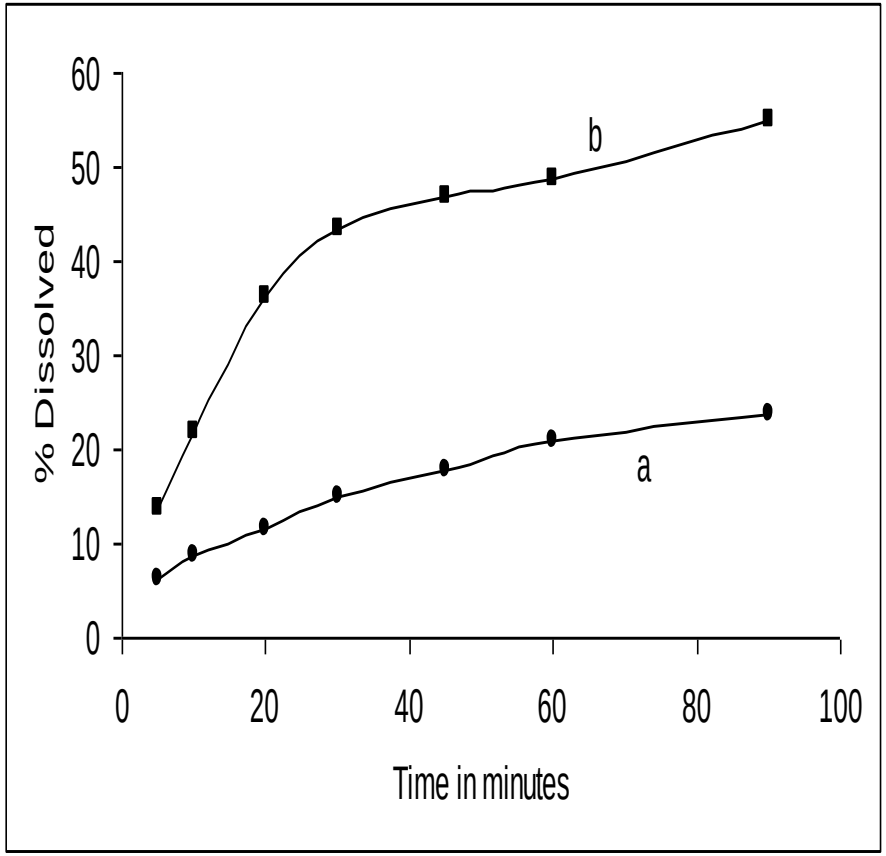
- **Standard curves were obtained by plotting the absorbance (OD) values at different concentrations (0-50 μg/ml) of each sample which gave rise to linear plots (y = mx+c). From these linear equations, the percentages of drug dissolved at different time were calculated by putting the OD values as 'y' and concentration of sample dissolved as 'x'. Then the dissolution profiles were obtained by plotting percentage of sample dissolved against time in minutes for the compounds and their inclusion complexes.**
- **The DE_{30} of each sample was calculated from the area under the dissolution curve at t = 30min (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time.**

$$DE_t = \frac{\int_0^t y_t \, dt}{y_{100}\, t} \times 100$$

yt = % of compound dissolved at time't'.
y100 = 100% dissolution at the same time.



Standard curve of acridone (a) & its inclusion complex (b



Dissolution profile of acridone (a) & its inclusion complex (b)

In vitro dissolution data

Sl. No	Sample	Percentage dissolved at different time intervals in min							DE_{30} %	$K_1 \times 10^{-2}$ min^{-1}
		5	10	20	30	45	60	90		
1	I	6.41	8.7	11.7	14.9	17.9	20.9	23.9	4.6	0.302
2	Ic	3.6	21.9	36.1	43.3	46.9	48.6	55.1	52.3	0.886
3	II	6.91	10.1	12.8	16.8	20.2	22.7	25.8	5.4	0.331
4	IIc	2.9	20.9	30.1	39.5	44.9	52.1	60.2	55.8	1.023
5	III	6.7	9.1	13.3	16.4	21.2	25.4	28.0	4.3	0.365
6	IIIc	11.9	20.2	29.9	46.6	47.5	52.3	58.8	51.1	0.985

In vitro dissolution data

Sl. No	Sample	Percentage dissolved at different time intervals in min							DE_{30} %	$K_1 \times 10^{-2}$ min^{-1}
		5	10	20	30	45	60	90		
7	**IV**	**8.7**	**12.1**	**15.4**	**18.7**	**23.2**	**27.6**	**32.1**	**6.8**	**0.431**
8	**IVc**	**12.2**	**24.5**	**45.9**	**56.3**	**61.1**	**65.1**	**75.4**	**76.8**	**1.559**
9	**V**	**5.1**	**7.6**	**10.3**	**12.9**	**17.1**	**22.8**	**28.2**	**6.6**	**0.368**
10	**Vc**	**8.2**	**16.2**	**24.9**	**34.2**	**43.4**	**52.5**	**63.2**	**68.7**	**1.112**
11	**VI**	**4.5**	**5.7**	**8.9**	**12.4**	**16.6**	**21.0**	**27.2**	**6.7**	**0.351**
12	**VIc**	**7.8**	**15.1**	**27.4**	**38.4**	**46.2**	**53.0**	**60.3**	**67.8**	**1.026**

Increase of k_1 s by 2.93, 3.1, 2.7, 3.62, 3.02 and 2.92 fold
& by 11.4, 10.3, 11.9, 11.3, 10.4 and 10.1 folds for the DE_{30} values after inclusion complexation

ATR-FTIR

- ATR-FTIR is a convenient method to evaluate drug (guest) and SEβCD (host) solid-state interaction [2].
- The IR spectra of the inclusion complex depicted a decrease in intensity, alter, and disappearance of some distinctive IR bands of ILO.
- The interaction of ILO with SEβCD was confirmed by a significant shift of some distinctive bands of ILO.

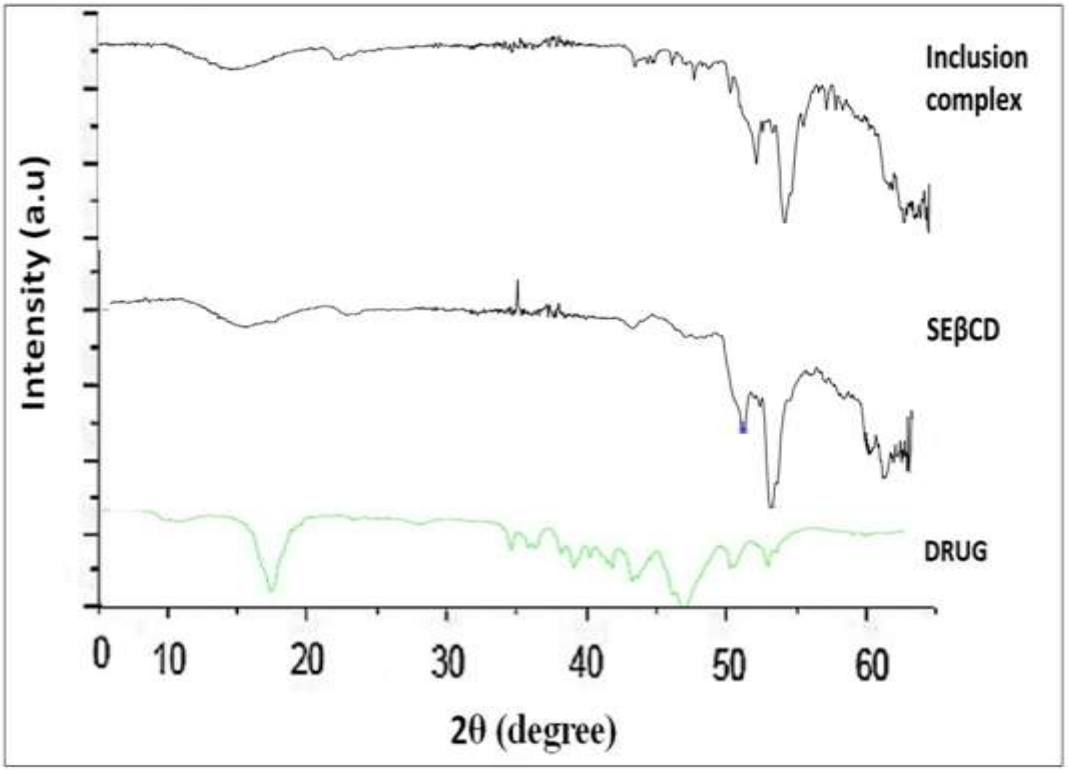


Figure 3. FT-IR spectra of Drug (ILO), SEβCD and inclusion complex

SVKM'S NMIMS Deemed-to-be UNIVERSITY

IECP 2020

DSC

- DSC of ILO displayed a sharp and well-defined endothermic peak at 120°C, conforming to the drug (ILO) melt point.
- In the endotherm of SEβCD, a broad peak was detected at 86°C. The endotherm of ILO was utterly disappeared into the stable inclusion complex.
- The amorphization of the drug describes an enhancement in the dissolution profile of ILO [3].

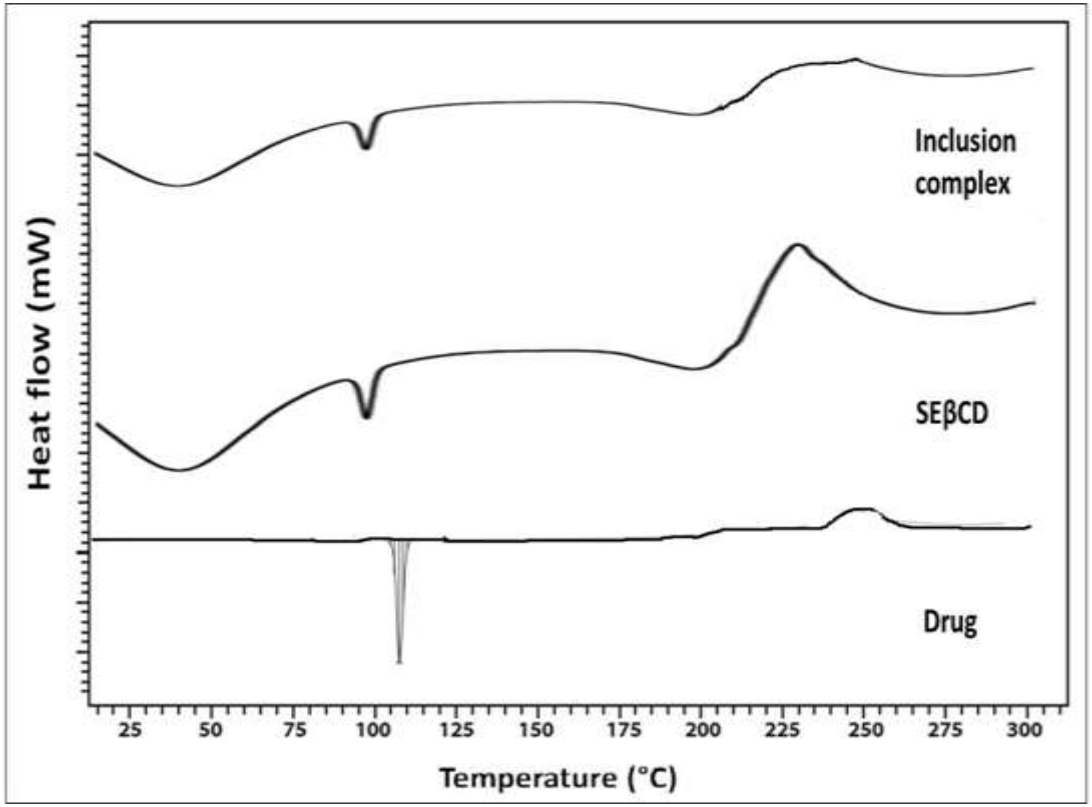


Figure 4. DSC thermogram of Drug (ILO), SEβCD and inclusion complex

SVKM'S NMIMS Deemed-to-be UNIVERSITY

IECP 2020

THE KNEADING TECHNIQUE

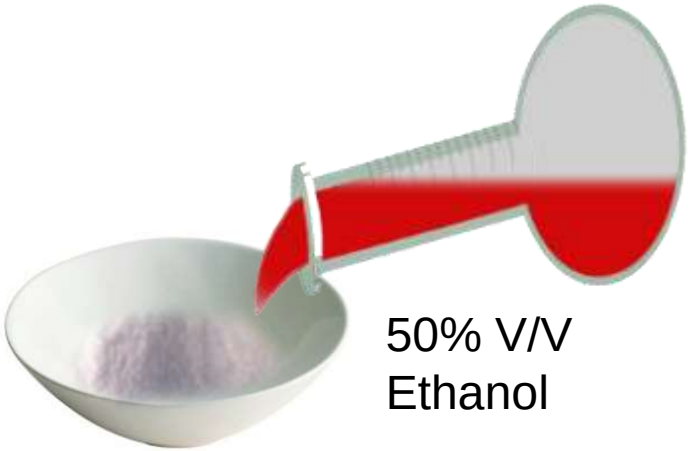
Inclusion complex of ß- cyclodextrin and the drug in 1:1, 1:2, 1:3 molar ratio can be prepared by the BCD, This method is based on impregnating the CDs with little amount of water or hydroalcoholic solutions to converted into a paste. 50% V/V ethanol were added while triturating to get slurry like consistency. Then slowly the drug was incorporated into the slurry, and trituration was continued further for 1 hrs. The slurry was then air dried at 25˚C for 24 hrs. Pulverized, and passed through sieve no. 100 and stored in a dessicator over fused calcium chloride.

Drug

Mixture

ß- cyclodextrin

Kneading Method……



50% V/V
Ethanol

Slurry
preparation

Oven or Air
drying

Drug-BCD
Powder

Co-precipitation technique

This method involves the co-precipitation of drug and CDs in a complex. In this method, required amount of drug is added to the solution of CDs. The system is kept under magnetic agitation with controlled process parameters and the content is protected from the light. The formed precipitate is separated by vacuum filtration and dried at room temperature in order to avoid the loss of the structure water from the inclusion complex.

Milling/Co-grinding technique

A solid binary inclusion compounds can be prepared by grinding and milling of the drug and CDs with the help of mechanical devices. Drug and CDs are mixed intimately and the physical mixture is introduced in an oscillatory mill or Ball mill and grinded for suitable time at suitable speed. Then it is unloaded, sieved through a 60-mesh sieve. This technique is superior to other approaches from **economic** as well as **environmental** stand point in that unlike similar methods it does not require any toxic organic solvents

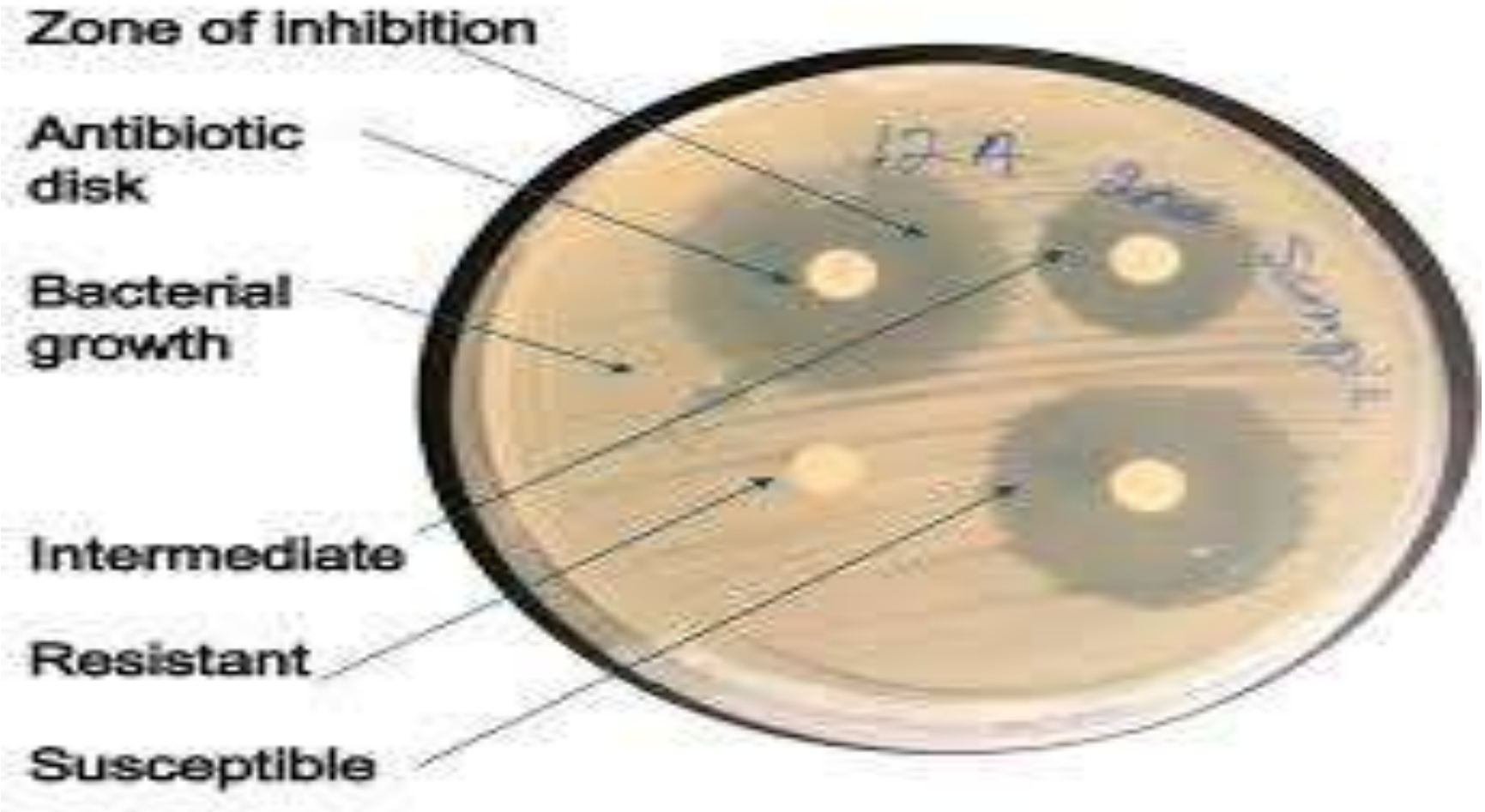
Other Methods.....

- Physical Blending
- Spray Drying
- Solvent Evaporation
- Freeze Drying
- Microwave Irradiation
- Supercritical Anti-solvent technique

CYCLODEXTRIN EFFECTS ON IMPORTANT DRUG PROPERTIES

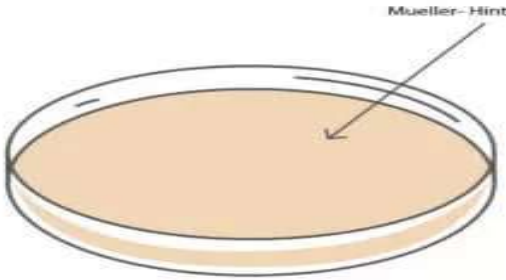
- Inclusion Complexation
- Solubility and dissolution enhancement
- Enhanced drug bioavailability
- Easier drug permeability
- Enhanced drug stability
- Improved drug safety
- Reduce gastrointestinal irritation, unpleasant smells/ tastes

KIRBY-BAUER TEST

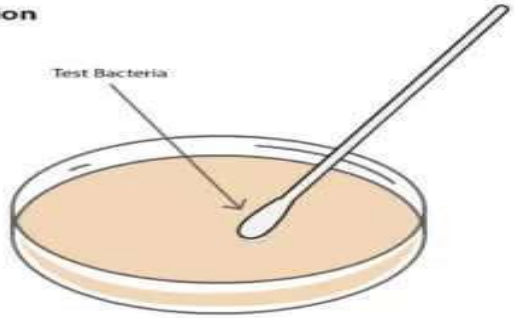


Zone Of Inhibition Test

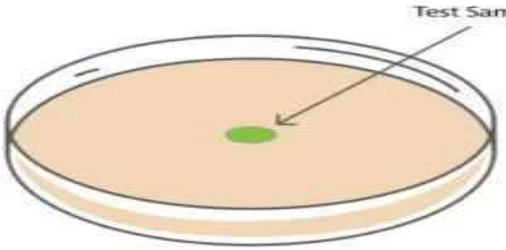
1. Preparation of growth medium



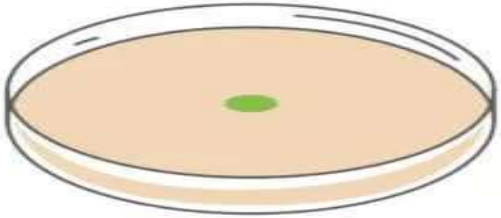
2. Inoculation



3. Insertion of antimicrobial sample

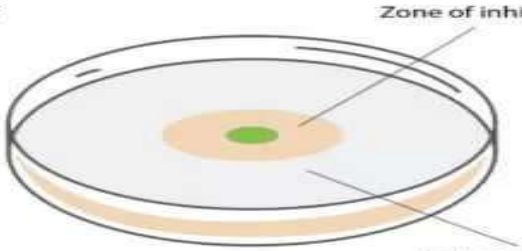


4. Incubation



Incubated for 18-24 hours at 36°C

5. Results



Treated products with strong antibacterial activity form a larger zone of inhibition or vice versa.

OK, now I understand your SIMPLE (???)explanation.

THANK YOU