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Studies of Inclusion Complexes of 4-Thiazolidinone Derivatives with β-Cyclodextrin Characterization, Phase solubility and inclusion mode

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ABSTRACT

4-Thiazolidinone and its derivatives attracted large attention owing to its diverse pharmaceutical applicability and usefulness for the society. In this context, two important 4-thiazolidinone derivatives namely 2-o-chloro phenyl 3-(benzothiazolyl-2') hydrazono-5-arylidene-4-thiazolidinone and 2-o-chloro phenyl 3-(benzothiazolyl-2') hydrazono-5-p-anisilidiene-4-thiazolidinone are synthesized from 2-hydrazinobenzothiazole taking as a origin material. However these compounds are less soluble and small degree of bio-accessible. To get rid out of the problem, inclusion complex of the compound has been prepared with β-cyclodextrin. From the changes in spectral characteristics, physical characteristics and antibacterial activities of the compounds and their respective inclusion complexes justify their formation. Thermodynamic parameters ΔG will predict the formation of inclusion complex. Presence of weak intermolecular forces in between host and guest acknowledged with the change in thermodynamic stability constant parameters.

Keywords: 2-Hydrazinobenzothiazole; Spectral; Weak intermolecular forces; Host

INTRODUCTION

In today's world, there is wide spread use of antibacterial drugs against bacterial infections. These types of antibacterial agents have activated the researchers to design and amend the present drugs to synthesize the alternate one. Strategies have been made to improve sensitivity and effectiveness of drug with the coming days. It is acknowledged that thiazolidinone and their derivatives paired with benzothiazolyl moiety are important pharmacophores that is present in various types of pharmaceutical agents. According to view of some researchers, all synthesized chemicals do not get a chance for being pharmaceutical agents because of their clinical failure in its infancy stage [1]. Similarly a drug cannot be successful in the market due to feeble pharmacokinetic properties to human beings, effects of drugs in negative way or due to insufficient clinical trial. So, the synthesized drug should show minor or zero toxicity for being heavy demand in the in the galaxy of medicines [2, 3]. Controlled drug delivery method should be adopted to eradicate the problem. Henceforth, the drug which is held by carrier molecule to be released in the body in a defined time and required place [4]. Above all, the effectiveness of the drug can be counted with its solubility in the available biological fluids in body. Different factors like adverse effects of drugs, enzymatic attack and change in PH value of body fluids can be determined in the controlled drug release method. Considering to above stated features, it is compelled us to select cyclo dextrin (CD) as a drug carrier molecule. Out of the different oligosaccharides available in nature, cyclo dextrin is one of them. It is cyclic and exits in three different forms like α-, β- and γ- . Glucose units of six to eight are present in different forms of cyclo dextrin in addition to the units are held by α-(1, 4) bonds. The uniqueness of cyclodextrin structure is the arrangement of hydrophobic cavity and hydrophilic structure that makes easier for formation of inclusion complex with different types of guests. In its structure, primary hydroxyl groups are present at the narrow end of the cone while secondary hydroxyl groups are present on the outermost side [5]. By this way the compound can be protected from environmental degradation besides increasing its aqueous solubility which will make it more effective by encapsulation with cyclodextrin. The most appropriate method for the solubility of weakly soluble drug is to make inclusion complex with cyclodextrin [6]. There is a development of solubility, tolerability and bio-availability nature of naked drug molecule after formation of inclusion complexes with β-cyclodextrin. Moreover our attention has been paid to develop a host-guest complex by combining 4-thilodinones derivatives with β-cyclodextrin. Based on cavity size either one or two hydrophobic molecule can be captured by one to three CDs molecules [7]. Taking into consideration of easily accessibility in markets, less toxic nature and economically viability forced us to select β-cyclodextrin for the formation of host molecules [8-10]. All these facts were motivating for us to develop some new inclusion complexes of 4-thiazolidinone derivative with wide structural variant. 4-Thiazolidinone is an organic compound where the fourth position is occupied by carbonyl

group in a five member ring containing sulphur and nitrogen and it is also one of the derivatives of thiazolidine heterocyclic compound. Multi-diversity behaviour and organic properties of 4-thiazolidinone leads to show various pharmaceutical activities like antimicrobial[11,12], antioxidant[13], anti-HIV [14], antihistaminic [15], anti-convulsant[16,17], anti-inflammatory [18-20]. Considering about the described matter, we have taken 2-hydrazinobenzothiazole taking as a starting material to prepare two compounds namely 2-o-chloro phenyl 3-(benzothiazolyl-2')hydrazono-5-arylidene-4-thiazolidinone and 2-o-chloro phenyl 3-(benzothiazolyl-2')hydrazono-5-p-anisilidiene-4-thiazolidinone respectively. Further their inclusion complexes were made with β-cyclo dextrin. Steps are also taken to know the physical and spectral characteristics of compounds and their respective inclusion complexes. In addition to its noteworthy parameters like thermodynamic stability constant, free energy change and antibacterial activities is also determined.

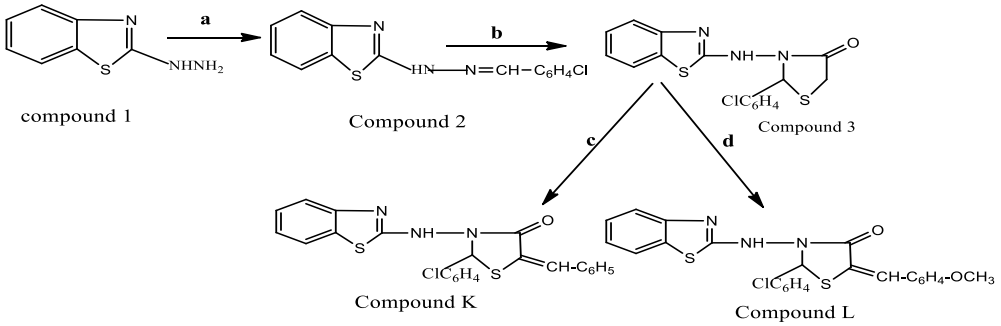
MATERIALS AND METHODS

Apparatus and Materials

The entire reagent used in the experimental procedure is acquired from Merck and Hi-Media. It belongs to analytical grade type and is used after purification. For dilution purpose double distilled water is taken and it is made in our laboratory. Electronic spectra of samples are taken with examining in Shimadzu UV-1700 spectrometer. IR Spectra are recorded by FTIR-8400S Shimadzu and its values are expressed in cm^{-1} with the help of KBr pellets. DRX-300 (300MHz) spectrophotometer instrument are used to scan ^1H NMR spectra ($CDCl_3$) and δ scale used to measure chemical shifts. To find melting point of compounds and its inclusion complexes, open capillary method is adopted. To check the purity of compounds sulphur detection is performed and TLC method using silica gel is done to check homogeneity.

Synthesis of Compounds and Preparation of Inclusion Complexes

The compounds were synthesized as per the method described in literature [21] in scheme-I



Scheme I: Synthesis of Compounds and Preparation of Inclusion Complexes

Where, a = ClC_6H_4CHO, C_2H_5OH, Piperdine
b = $SHCH_2COOH$, C_6H_6
c = C_6H_5CHO, CH_3COONa, CH_3COOH
d = $H_3COC_6H_4CHO$, CH_3COONa, CH_3COOH

Step-1

Preparation of Schiff's Base (Compound 2): A round bottom flask containing 20ml of ethanol is taken followed by addition of a mixture of 2-hydrazinobenzothiazole (1.65gm, 10 mmole) and o-chlorophenyl aldehyde (10m mole). Adding 4 to 6 drops of piperdine to the above mixture refluxing is carried on a water bath for a time period of 4 hours. Excess of solvent was poured in crushed ice. By filtrating, drying and recystalising solid is obtained. M.P.220^oC, yield-2.2gm (70%), (Found S, 12.11%, $C_{14}H_{10}N_3SCl$ requires S, 12.16%).

Step-2

Synthesis of 3-(Benzothiazolyl-2') hydrazono-2- o-Chlorophenyl-thiazolidinone(Compound 3):Mercaptoacetic acid (0.1 gm,1m mole) and solution of the hydrazono derivative of benzaldehyde (0.26 gm,1 m mole) are taken in dry benzene (15 ml) in a round bottom flask. The mixture in the flask stirred and refluxed for 6 hours and 4 hours respectively during which the preferred compounds are precipitated. The obtained compound was filtered, dried and recrystallized from ethanol. M.P. 217^oC, yield-0.23 gm (68%), (Found: S, 19.59% $C_{16}H_{12}N_3S_2OCl$ requires S, 19.57%).

Step-3

Synthesis of 2-o-chloro phenyl 3-(benzothiazolyl-2')hydrazono-5-arylidene-4-thiazolidinone (Compound-K): Equimolar amount of 3-(benzothiazolyl-2')hydrazono-2-o-chlorophenyl 4-thiazolidinone (0.32 gm, 1 m mole) and benzaldehyde (0.1 gm, 1 m mole) mixed with fused sodium acetate (0.2 gm) in glacial acetic acid (10ml) was heated under reflux for 4 hours. The extra amount of solvent was distilled off and the remaining residue was transferred to ice cold water to get a solid of yellow colour. The compound is filtered, washed in water, dried and recrystallized from ethanol. The obtained compound is K. Its melting point is 225^oC, yield is 0.24 gm (60%), (Found: S, 18.20% $C_{23}H_{16}N_3S_2OCl$ requires S, 18.18%). Following same procedure compound 2-o-chloro phenyl 3-(benzothiazolyl-2') hydrazono- 5-p-anisilidiene-4-thiazolidinone (**L**) is prepared but p-anisaldehyde is taken in place of benzaldehyde during the last step.

Step 4

Aqueous Phase Solubility Study: Higuchi Connors method [22] has been used for aqueous phase solubility study of two different synthesized

compounds K and L at various conc. of β-cyclodextrin. As per the method, precise amount of substance are taken in a conical flask containing β-cyclodextrin in varied concentration starting from 0mM to 7mM. The whole solution has shaken in a rotary flask shaker at the then prevailing temperature for 48 hours till attaining the equilibrium. These solutions are then filtered by whatmann filter paper No-1 and analyzed by UV visible spectrometer having the range of 200-400nm.To finish the process, optical densities of seven different solutions are measured with respect to their absorption maxima λ_{max} and the graph is plotted between optical densities against different concentrations of β-cyclodextrin as given in (fig 1).

Step 5

Preparation of Inclusion Complexes: Co-precipitation method [23-25] is adopted here because of water insolubility nature of developed drug although different methods are available for preparation of inclusion complex. Emphasizing over all the above information's, inclusion complexes of compound K and L are prepared. For carrying the process, requisite conc. of compound solution are made and added with prepared solution of β-cyclodextrin. At room temperature the resultant mixture is stirred for 48 hours followed by filtration. Further the filtrate is cooled in a refrigerator for 48 hours. The precipitate washed with distilled water and dried it for another 24 hours.

Step 6

Evaluation of Antibacterial activity: The study of antibacterial activities of compounds and inclusion complex has been performed by using cup plate method [26]. According to the method, Dimethyl sulphoxide with strength of 500µg/ml were taken and solution of compounds as well as inclusion has been prepared with same concentration. Then two bacteria *E.coli* and *S.aureus* were inoculated into 100ml of the sterile nutrient broth. Further it has been incubated at a temperature of approximately 37 $^{\circ}$C for 24 hours. Standardized diameter of agar plates were used to inoculate them one after other with the test organisms aseptically. Micropipette is used to take the drug, test solutions in a plate and then the plates were kept inside the refrigerator for 2 hours with a temperature maintaining at the range of 8-10 $^{\circ}$C for right dispersal of drug into the media. Further transferring the petri plates into incubator with a temperature of nearly 38 $^{\circ}$C for 22 hour. Then Zone of inhibition can be found by using venire scale in the Petri plates and data is presented in tabular form in Table 2.

RESULTS AND DISCUSSION

Studies of Phase Solubility Diagram

The formation of inclusion complexes between β-cyclodextrin (β-CD) and synthesized compound of 4-thiazolidinone derivative acting as host and guest respectively can be ascertained from their phase solubility studies. Inclusion complexes created by β-CD in aqueous solution where water molecules taken by host molecules bears hydrophobic in nature. However, the hydroxyl groups which are present in the outer sphere of β-CD may combine with extra molecule or same molecule. An inclusion and non-inclusion complex in a saturated aqueous solution is formed with drug molecules at a time in cyclodextrin [27]. Therefore, the equilibrium constant value is depending on concentration and experimental method used. In the same way temperature controls host-guest interaction besides combination of drugs with various cyclodextrin. The host molecule bearing hydrophobic cavity offer guest molecule suitable environment for formation of host-guest complex. The outside of the host molecule with water forms hydrogen bonding instead of avoiding for the association of guest molecule by which net volume decreases. Furthermore, with increasing temperature hydrophobic interaction between host-guest decreases which leads to increase of partial volume. Interaction of hydrophobic part of host molecule into cyclodextrin cavity and dehydration of guest molecules are responsible for formation of host-guest complex as per the thermodynamic view-point. The formation of hydrogen bonding is one of the reasons for stability of complex. In addition to above facts conformational changes and water molecule elimination also lead for the formation of host-guest complex [28, 29]. Inclusion complex formation in the solution state can be determined by phase solubility diagram. It is determined as indicated by phase solubility diagram profiles based on solution capability of substrate [30]. Basically, phase diagram is grouped into 2 types such as A and B. Group A is once again classified into three subgroup namely A_L, A_P and A_N. In all these subgroups different relation of guest solubility with respect to β-CD concentration takes place. The guest solubility makes linear relation with cyclodextrin concentration in A_L type of phase diagram whereas in A_P and A_N types deviation takes place from either side of the straight line. Stoichiometry relation between compound of 4-thiazolidinone derivative with β-CD results 1:1 ratio which justify the formation of A_L type diagram. From the figure 1 and 2 phase solubility plots of compounds K and L are observed in addition to linear increase of solubility of these compounds with rising conc. of β-CD. At higher conc. of β-CD small negative deviation is found. In view of the above fact that the slopes of all plots are taking away less than one signifies stochiometric of inclusion complexes is 1:1 as described by Z.Szelti [31].

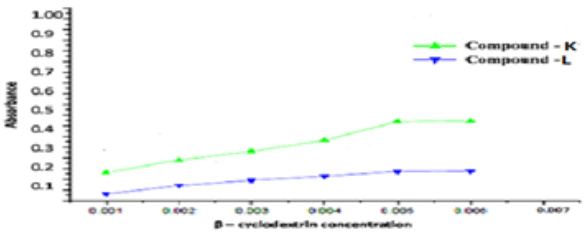


Figure 1: Variation of abs. with conc.

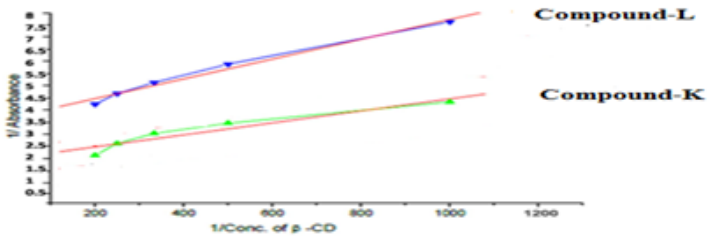


Figure 2: Variation of 1/ abs. with 1/conc.

Studies of Thermodynamic Properties

In order to discuss the thermal nature of inclusion complexes, its stability constant (K_T) can be determined by referring Benesi-Hilderband relation [32].The equation representing the relation is $1/\Delta A = 1/ \Delta Є + 1/ K_T [Guest]_o \Delta Є.[\beta\text{-}CD]_o$, where ΔA and $\Delta Є$ is alteration in absorption and molar extension coefficient, $[Guest]_o$ is concentration of compound in inclusion complex and $[\beta\text{-}CD]_o$ is molar concentration of β-CD. On drawing a plot of $1/\Delta A$ verses $[\beta\text{- CD}]_o$ for compounds, good linear correlations were arrived. By use of relation K_T = Intercept/Slope, K_T values for all the complexes were found. The K_T values of K and L inclusion complexes were found to be 802.21 and 894.17 M^{-1} respectively. It is found to be remaining in the range of 100 to 1000 M^{-1} indicating substantial stabilities. The interaction between 4-thiazolidinone derivatives with β-cyclodextrin rests upon the factors like hydrogen bonding, vander waal's force and hydrophobic interaction between host-guest molecule [33,34]. The free energy of activation of inclusion complexes of compound K and L obtained as -15.028 and -15.881 kJ/mole value after calculation. The change in Gibb's free energy (ΔG^0) is the criteria to do some useful work having negative value and should be spontaneous process at constant pressure and temperature [35]. The change in free energy of inclusion complexes is calculated as i.e. $\Delta G = -2.303RT \log K$ at 25^0C where K is equilibrium constant. Since change in free energy is negative, so the process is thermodynamically allowed.

Studies of Physical and Spectral Characterisation

With the host-guest complex formation leads to an inclusion complex which results in alternation of colour and melting point of the compound. The colours of compound K and L are found brown and brownish white, whereas the formed inclusion complexes are light brown and reddish white. The compound K and L bears a melting point of 225°C and 232°C respectively whereas inclusion complexes are 229°C and 237°C respectively. The extra heat is required to bring the molecules out of β-cyclodextrin cavity and it can be confirmed with the change in melting point from compound to inclusions. The quantity of inclusion complex formed is less as compared to original compounds**.** The change in spectral characteristics of compound K and L with their inclusion is given in the table 1. From the IR data, it is found that the compounds and their inclusion are found to be observed at suitable frequency range. Further IR spectra of compounds changes after the development of the inclusion complex. All these changes signify the transference of complexes into the cavity of β-cyclodextrin and creation of weak forces like hydrogen bond, vandar walls force and hydrophobic interaction between host and guest molecules [33-35]. It can be interfered that the δ values of the inclusion complexes are having dissimilar value on contrast with relevant compounds. This indicates PMR signal are changed due to formation of inclusion complexes which could be endorsed due to encapsulation induced shielding within the cavity of β-cyclodextrin (Figures 3-6).

Table 1: Spectral data of synthesized compounds and their inclusions**.**

Compound/ Inclusion complex	**$UV_{\lambda max}$**	**IR(KBr) cm^{-1}**	**^{1}H NMR**	**Elemental Analysis Calculated (Found)**		
				C	**H**	**N**
Comp –K	277	655.80(C-Clstr),738.74(C-Sstr),1367.53(C-Nstr),1573.91,1510.26(C=CStr), 1610.56(C=Nstr),1658.78(C=Ostr),3190.26(N-H str)	^{1}H NMR ($CDCl_3$) : δ 7.26-8.25 (d, 6H, Ar-H), 4.62(s,1H,C-NH),7.50(s,1H,C-H),7.3-7.8 (t,8H, Ar-H)	61.39 (61.25)	3.58 (3.35)	9.33 (9.13)
Comp –K with β-CD	267	705.95(C-Clstr),734.88(C-Sstr),939.33(N-C-Sstr),1321.24(CNstr),1512.19(C=CStr),1610.56(C=Nstr),3217.27(N-Hstr)	^{1}H NMR ($CDCl_3$): δ 7.21-8.03 (d, 6H, Ar-H), 3.59 (s,1H,C-NH),7.28(s,1H,C-H)			
Comp –L	273	659.66(C-Clstr),705.96(C-Sstr),1321.26(C-Nstr),1510.26(C=CStr),1610.56(C=Nstr),2929.87(Ar-Hstr),3224.89(N-H str)	^{1}H NMR ($CDCl_3$):δ 7.18-8.45 (d, 6H, Ar-H), 3.86 (s,1H,C-NH),7.32(s,1H,C-H),6.94-8.08 (t,8H, Ar-H)3.86 (s,3H,OCH_3)	60.05 (60.15)	3.77 (3.87)	8.75 (8.85)

Comp –L with β-CD	265	674.01(C-Clstr),736.31(C-Sstr),945.12(N-C-Sstr),1510.26(C=CStr),1610.56(C=Nstr),1658.78(C=Ostr),2929.87(Ar-Hstr),3201.83(N-H str)	^{1}H NMR ($CDCl_3$):δ 7.18-8.44 (d, 6H, Ar-H), 3.87 (s,1H,C-NH),7.28(s,1H,C-H),6.69-7.71 (t,8H, Ar-H)3.87 (s,3H,OCH_3)	

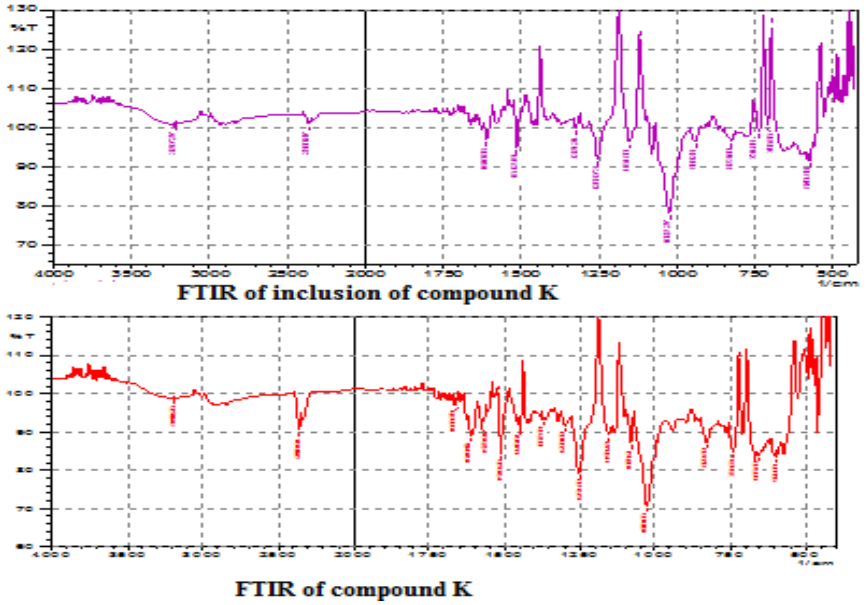


Figure 3: FTIR spectra of compound K (Lower) and its inclusion (Upper)

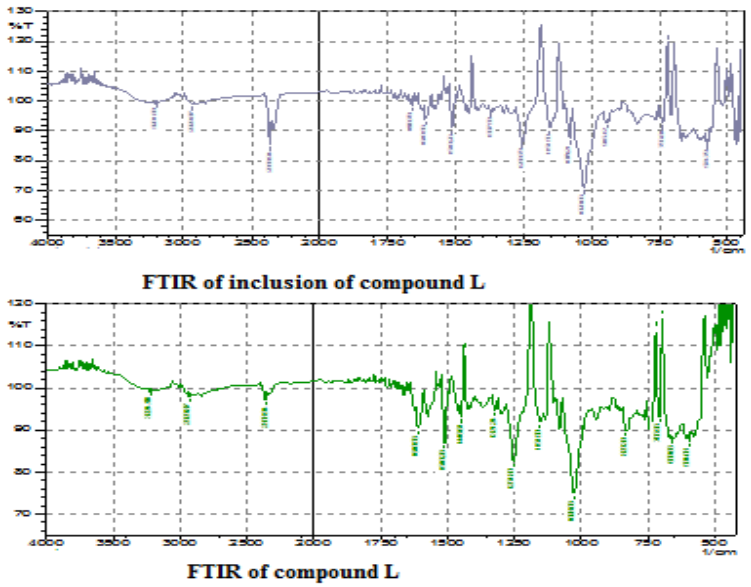


Figure 4: FTIR spectra of compound L(Lower) and its inclusion (Upper)

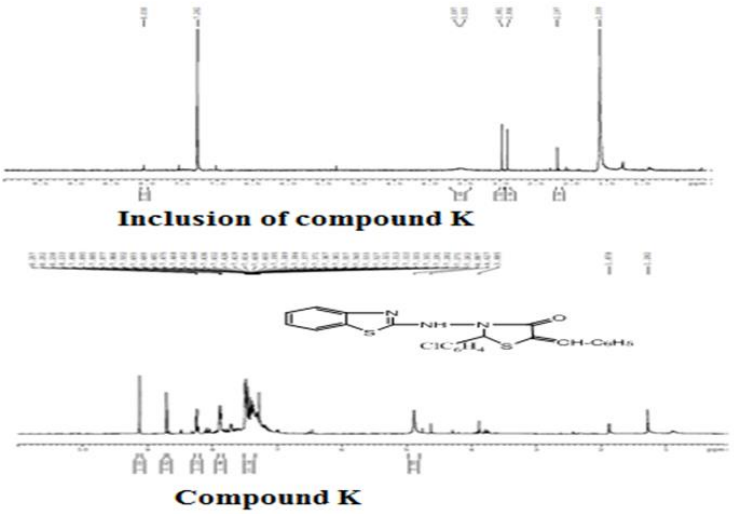


Figure 5: ^{1}H NMR of compound K and its inclusion

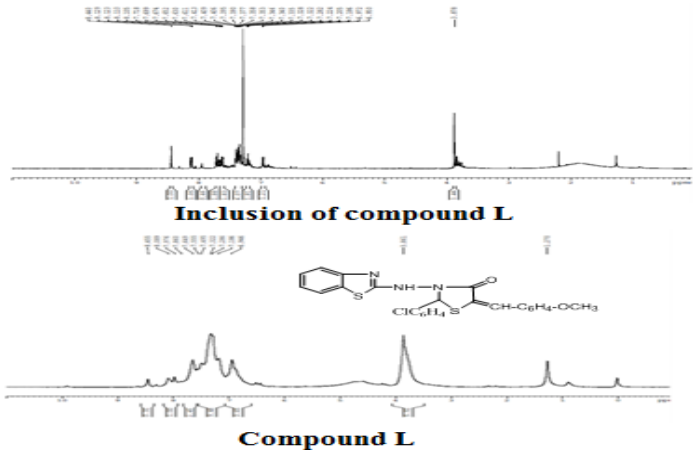


Figure 6:^{1}H NMR of compound L and its inclusion

Studies of Antibacterial activities of compounds and their inclusion complexes: The study of antibacterial activities of compounds and inclusion complex against two bacterial strains *E.coli* and *S.aureus* establish the fact that the diameter of the zone of inhibition of inclusion complexes obviously more as compared that of the compounds as shown in the table 2. Out of all the substances undergone the test, the inclusion complex of compound-L shows maximum activity against *S.aureus* at the same time inclusion complex of compound-K shows maximum activity against *E.coli*. The significant improvement of antibacterial activity of the inclusion complexes is on account of their solubility in the aqueous medium which makes them more bio-accessible and more effective towards specific tissues as a result their drug efficiency increases. This happens due to higher solubility of the compounds due to inclusion complex formation there by leading to rise in bio accessibility.

Table 2: Antibacterial studies of the compounds and their inclusion complexes

Sl.No.	**Compounds/complexes**	**Diameter of zone of inhibition (mm)**	
		E. coli	***S.aureus***
1.	Compound K	09	12
2.	Inclusion complex of comp. K	12	14
3	Compound L	10	11
4	Inclusion complex of comp. L	13	13

CONCLUSIONS

In this review, we summarise the research that the bio accessible and soluble nature of the compound enhanced after formation of inclusion complexes. Indeed it is confirmed that there is existence of weak forces that leads to bind host and guest molecules. Apart from this, there is

thermodynamic acceptance between compound and β-cyclodextrin. With the formation of complex, there is enhancement of therapeutic potential as well as antibacterial activities of the synthesized drugs. Proper molecular interaction has also recommended reasonable extent of progress in the formation of inclusion complex.

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CONFLICT OF INTEREST

The authors declared no conflict of interests.

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