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## **Studies on inclusion complexes of substituted indole derivatives with activating and deactivating group**

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### **ABSTRACT**

*Indole and its analogues are very good pharmacophores exhibiting wide spectrum of fungicidal, bactericidal and tuberculostatic activities. These have poor solubility in polar medium which may be a limiting factor reducing their bio-accessibility. The solubility of these compounds in aqueous medium (polar medium) can be enhanced by forming inclusion complexes with a non-toxic oligosaccharide, namely  $\beta$ -cyclodextrin. In this paper an attempt has been made to synthesise some [arylideneamino]-1,3,4-thiadiazino[6,5b]indoles with activating (Hydroxy) and deactivating (Nitro) groups in their purest form and then to prepare their respective inclusion complexes with  $\beta$ -cyclodextrin. The formation of compounds and their inclusion complexes have been ascertained from elemental analysis and study of spectral characteristics. Thermodynamic properties of the inclusion complexes are also studied to know whether the formation of inclusion complex is thermodynamically stable or not. In addition, the study of thermodynamic properties also reveal the type of interaction existing in between the host  $\beta$ -cyclodextrin and the compound concerned.*

**Key words:** Indole, [Arylideneamino]-1,3,4-thiadiazino[6,5b]indoles,  $\beta$ -Cyclodextrin, Inclusion complex.

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### **INTRODUCTION**

Indole and its derivatives are very good pharmacophores exhibiting wide spectrum of pharmacological activities such as antidepressive, anti-inflammatory, anti-fungicidal, anti-bactericidal and anti-tuberculostatic activities [1-4]. Azaediones and thiazolidinones show excellent antimicrobial activities [5-8]. There are also reports that compounds containing indole

or substituted indole coupled with azedinone or thiazolidinone units are acting as drugs for treating a number of diseases[7,8]. Since the bio-accessibility of drugs depend upon their solubility, the poor solubility of these compounds in polar medium (in water) may be a limiting factor reducing pharmacological activities. The solubility and bio-accessibility of these compounds can be enhanced significantly by forming inclusion complex with cyclodextrins[9]. Out of all the known cyclodextrins,  $\beta$ -cyclodextrin is usually considered for inclusion complex formation because it is cheaper, easily available and highly stable towards heat and oxidation[10,12]

In the present work an attempt has been made to synthesize some 2-[arylidenamino]-1,3,4-thiadiazino[6,5b]indoles in their purest forms starting from indole-2,3-dione. The aryl aldehydes considered in the synthesis are of three types containing activating (-OH) and deactivating (-NO<sub>2</sub>) group at ortho position. The inclusion complexes of the compounds have been prepared with  $\beta$ -cyclodextrin. The formation of compounds and their inclusion complexes have been ascertained from elemental analysis, melting point data and study of spectral characteristics. Thermodynamics properties of the inclusion complexes are also studied to know thermodynamic stability of inclusion complexes and the type of interaction in between the host and guest.

## EXPERIMENTAL SECTION

### Apparatus and Materials

All the chemicals of acceptable standards were procured from local market. Double distilled water to be used as solvent was prepared in the laboratory. Electronic spectra were recorded on Shimadzu UV-1700 Spectrophotometer and IR spectra were recorded in KBr pellets in Shimadzu 8400 FTIR Spectrophotometer. Melting points were recorded by open capillary method.

### Synthesis of 2-[Arylidenamino]-1, 3, 4-thiadiazino [6,5b] indoles

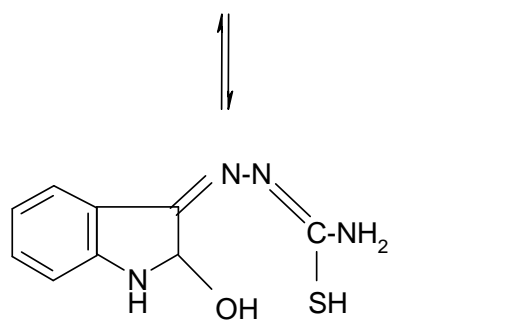
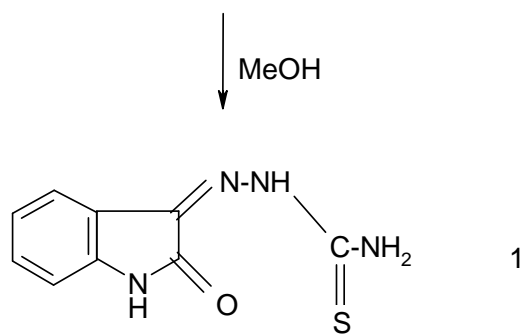
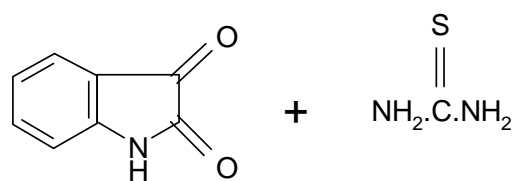
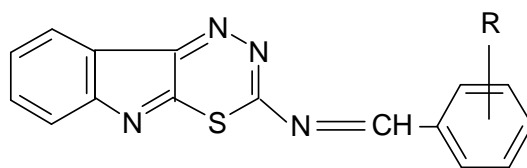
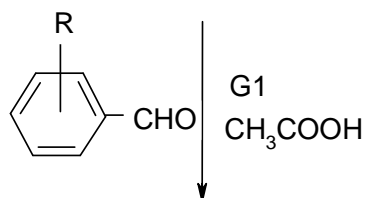
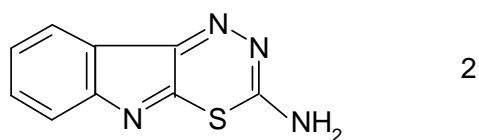
Three different 2-[arylidenamino]-1, 3, 4-thiadiazino [6,5b] indoles were synthesized starting from indole -2,3--dione (as per the scheme-I) through the following intermediate steps[8]

i) Synthesis of 3-Thiosemicarbazideindole-2-one: A mixture of 2gm of indole-2, 3-dione and 1.23gm of thiosemicarbazide in 50 ml of methanol was refluxed for one hour. The completion of the reaction was checked by TLC. The excess of methanol was distilled out. The content was cooled and poured into ice cold water. It was filtered, washed with water, dried and recrystallised from ethanol to obtain 3-Thiosemicarbazideindole-2-one[8]

ii) Synthesis of 2-Amino-1,3,4-thiadiazino[6,5-b] indole: 3gm of 3-Thiosemicarbazideindole-2-one was mixed with small quantity of cold and concentrated H<sub>2</sub>SO<sub>4</sub>. The reaction mixture was left at room temperature for 16 hours. The reaction mixture was then poured into ice-cold water and neutralized with liquid NH<sub>3</sub> to obtain a solid mass. The solid mass was filtered by using Whatmann-42 filter paper. It was washed with water, dried and recrystallised from ethanol to yield 2-Amino-1, 3, 4-thiadiazino [6, 5-b] indole.

a) Synthesis of Benzylidenamino-1, 3, 4-thiadiazino [6,5b] indole (Compound-I): 1.06gm of Benzaldehyde and 2.02gm of 2-Amino-1, 3, 4-thiadiazino [6, 5-b] indole were taken in 50ml of methanol. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered, washed with water and dried. The dried mass was recrystallized from ethanol.

Scheme-I

Cold Conc. H<sub>2</sub>SO<sub>4</sub>R = H, o-NO<sub>2</sub>, o-OH

b) Synthesis of 2-[2-Nitro benzylidenamino]-1, 3, 4-thiadiazino [6,5b] indole (Compound-II): 1.51 gm of o-Nitrobenzaldehyde and 2.02gm of 2-Amino-1, 3, 4-thiadiazino [6, 5-b] indole were taken in 50ml of methanol. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered and washed with water and dried. The dried mass was crystallized from ethanol.

c) Synthesis of 2-[2-Hydroxy benzylidenamino]-1,3,4-thiadiazino[6,5b] indole (Compound-III): 1.22 gm of Salicylaldehyde and 2.02gm of 2-Amino-1, 3, 4-thiadiazino [6, 5-b] indole were taken in 50ml of methanol. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered and washed with water and dried. The dried mass was crystallized from ethanol.

#### Phase Solubility Measurements:-

The aqueous phase solubility of the compound at various concentration.  $\beta$ -cyclodextrin (0-10mM) was studied by Higuchi-Corner method[13]. Accurately weighed sample of these compounds was shaken in rotary flash shaker at room temperature in a series of conical flask for a period of 48 hours till the attainment of equilibrium. The solutions were filtered through whatmann-42 filter paper and were analyzed in a UV-visible spectrophotometer. The various values of absorbance at  $\lambda$ -max were plotted against different concentrations of  $\beta$ -cyclodextrin.

#### Synthesis of inclusion complexes:-

The inclusion complexes of the compounds (I,II and III) with  $\beta$ -cyclodextrin were prepared as per co-precipitation method[14]. The solutions of these compounds in required concentrations were added drop by drop to  $\beta$ -cyclodextrin solution of the required concentration. The mixtures were stirred for a period of 48 hours and filtered. The filtrate was cooled for 24 hours in refrigerators. The precipitate obtained was filtered through G-4 crucible, washed with water and dried in air for 24 hours.

#### Study of thermodynamic properties:-

The thermodynamic stability constant of the complexes was calculated using Benesi-Hilderband relation[15]. The stability constant K of each complex was calculated with increasing temperature. From the slope of the linear plot of  $\ln K$  vs.  $1/T$ ,  $\Delta H$  was calculated. Then  $\Delta S$  was calculated from vant Hoff's equation

$$\ln K = \frac{\Delta H}{RT} - \frac{\Delta S}{R}$$

The value of  $\Delta G$  was calculated at 298 K using the equation:

$$\Delta G = -RT \ln K$$

## RESULTS AND DISCUSSION

The synthesis of Compound-I (Benzylidenamino-1,3,4-thiadiazino[6,5b]indole), Compound-II (2-[2-Nitro Benzylidenamino]-1,3,4-thiadiazino[6,5b] indole) and Compound-III (2-[2-Hydroxy Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole) have been confirmed from elemental analysis and IR data as shown in Table-1. The elemental composition matches with theoretical data. IR data of the compound-I show characteristic absorption at 672,1296,1611,1682 and 3141  $\text{cm}^{-1}$  indicating the presence of C-S, C-C, N-N, C=N and benzene ring in the compound as expected. IR-data of the compound -II show characteristic absorptions at 719, 1301, 1462, 1581, 1701 and 3146  $\text{cm}^{-1}$  indicating the presence of C-S, C-C, C-N, N-N, C=N and benzene ring in the compound as expected. Similarly, the IR-data of the compound -III show characteristic

absorptions at 672, 1294, 1611, 1683 and 3142  $\text{cm}^{-1}$  indicating the presence of C-S, C-C, N-N, C=N and benzene ring in the compound.

**Table-1: Analytical data of Compounds with and without inclusion complex**

*Compound-I : Benzylidenamino-1,3,4-thiadiazino[6,5b]indole*  
*Compound-II : 2-[2-Nitro Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole*  
*Compound-III : 2-[2-Hydroxy Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole*

S. No.	Compound/Complex	Melting Point	Colour	Elemental Analysis (First line indicates finding value & second line indicates calculated value)					$\lambda$ max ( $\text{\AA}^0$ )	IR( KBr ) $\text{cm}^{-1}$
				C	H	N	S	O		
1	Compound-I	224	Yellow	66.4 66.2	3.45 3.44	19.4 19.3	1.0 1.03	O	3550	672(C-S) 1296(C-C) 1611(N-N) 1682(-C=N) 3141(Ring)
2	Compound-I- $\beta$ - CD	228	Whitish Yellow	-	-	-	-	-	3542	670(C-S) 1290(C-C) 1605(N-N) 1679(C=N) 3130(Ring)
3	Compound-II	230	Yellow	57.5 57.3	2.7 2.69	20.9 20.9	9.56 9.55	9.55 9.55	3560	719 (C-S) 1301(C-C) 1462 (C-N) 1581(N-N) 1701(-C=N) 3146(Ring)
4	Compound-II- $\beta$ - CD	236	Pale Yellow	-	-	-	-	-	3551	717 (C-S) 1298(C-C) 1460(C-N) 1576(N-N) 1698(-C=N) 3138(Ring)
5	Compound-III	239	Yellow	62.8 62.7	3.3 3.26	18.4 18.3	10.5 10.4	5.0 5.2	3540	672(C-S) 1294(C-C) 1611(N-N) 1683(C=N) 3142(Ring)
6	Compound-III - $\beta$ - CD	246	Whitish Yellow	-	-	-	-	-	3530	669(C-S) 1290(C-C) 1610(N-N) 1679(C=N) 3130(Ring)

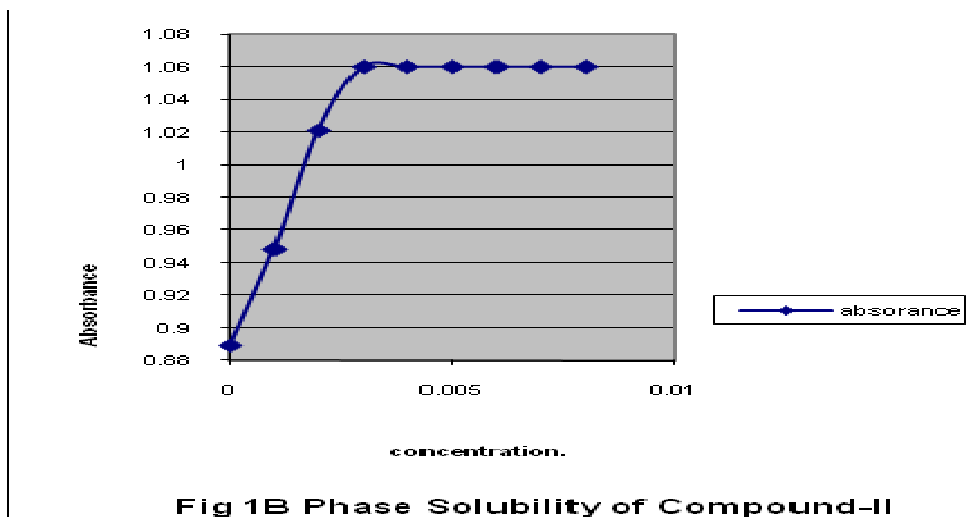
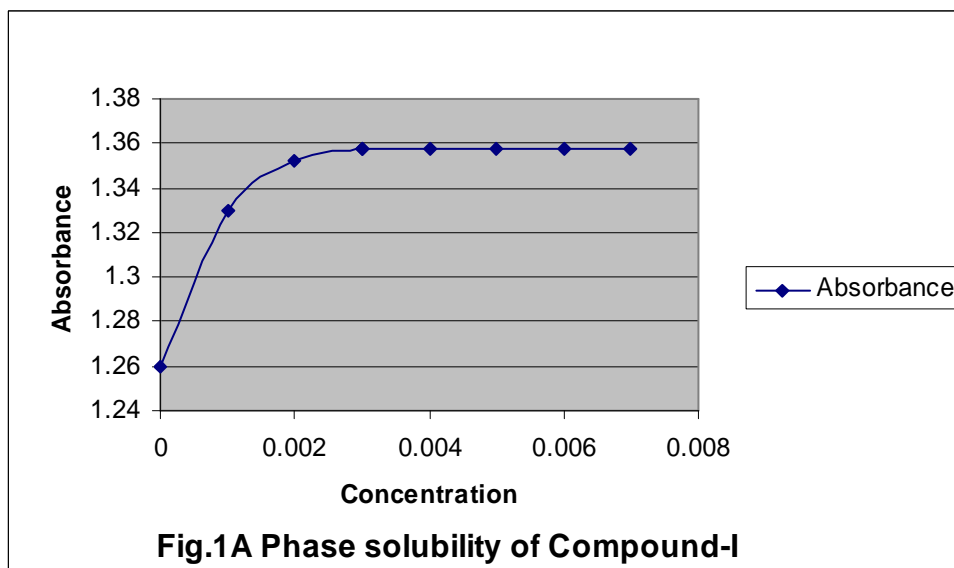
**Table-2: Thermodynamic data of inclusion complexes at 298 K**

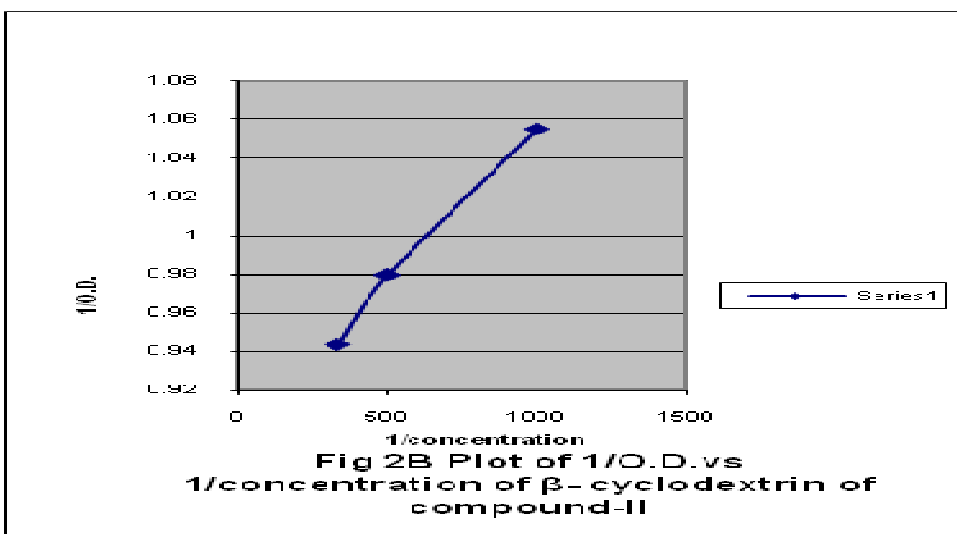
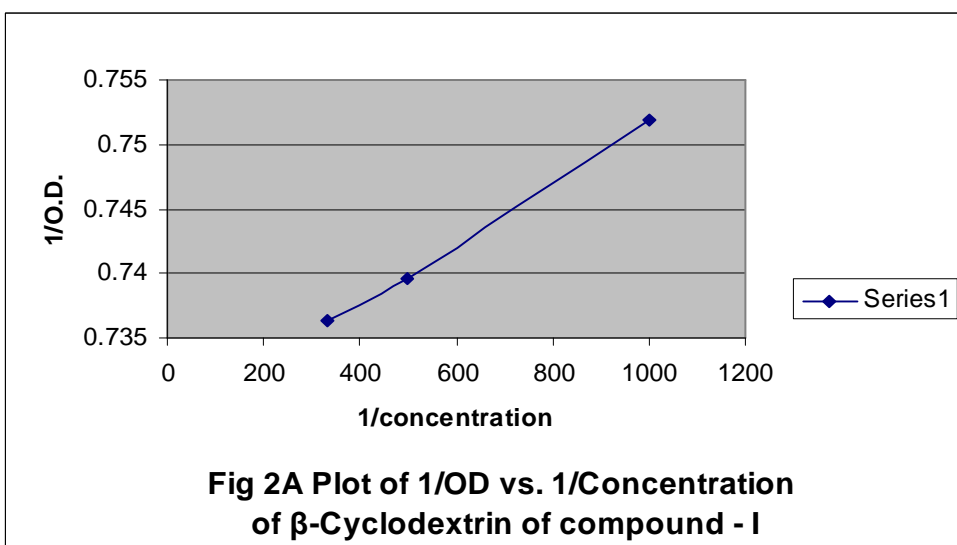
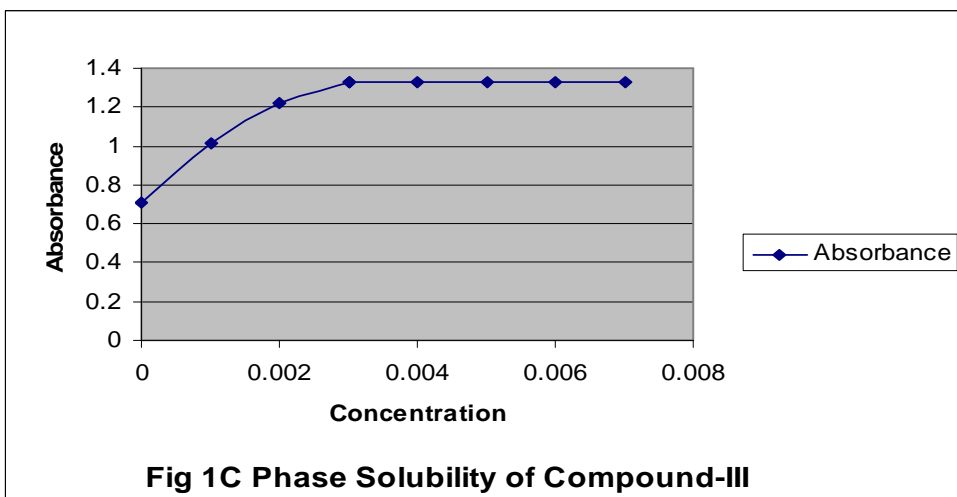
*Compound-I : Benzylidenamino-1,3,4-thiadiazino[6,5b]indole*  
*Compound-II : 2-[2-Nitro Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole*  
*Compound-III : 2-[2-Hydroxy Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole*

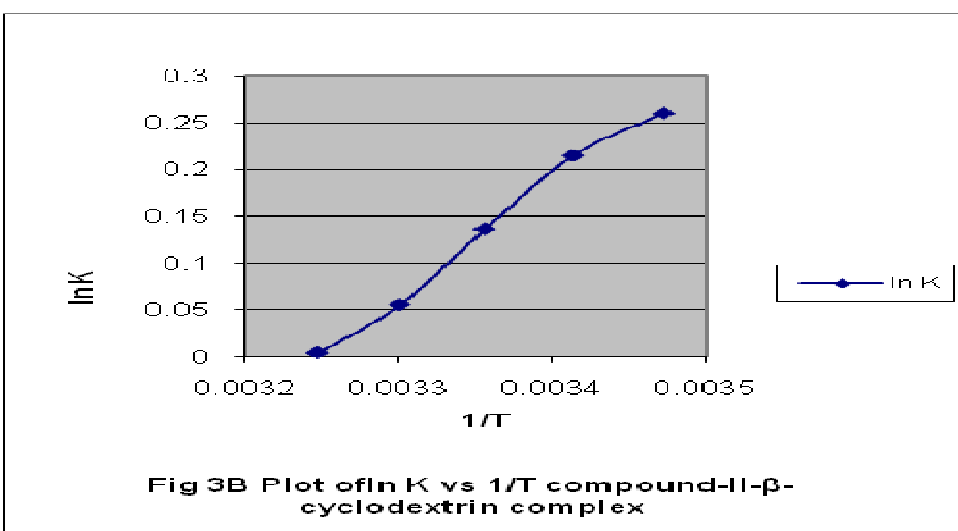
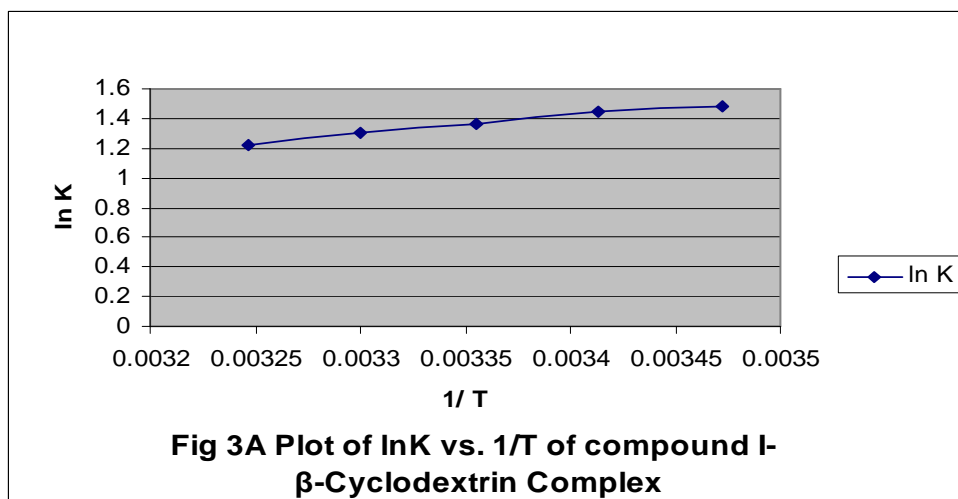
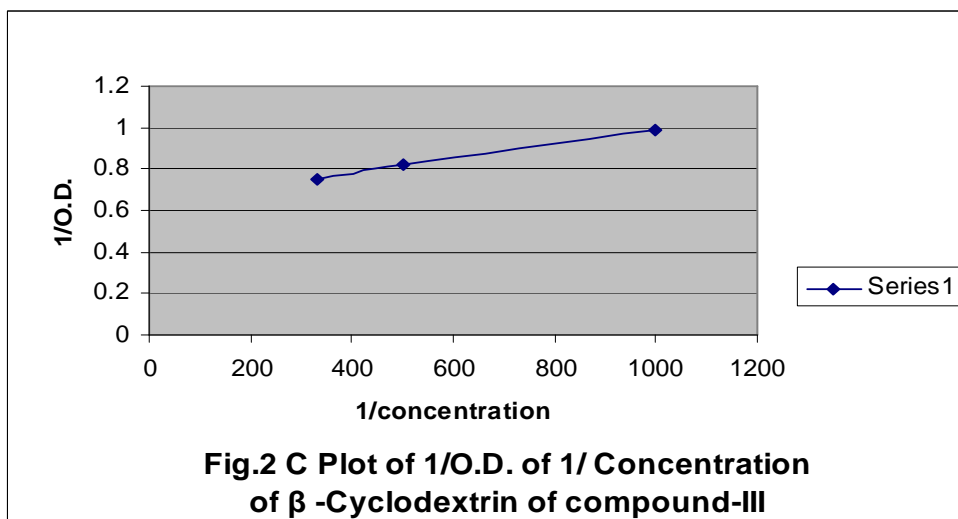
Complexes	K( $\text{M}^{-1}$ )	$\Delta G$ (KJ/MOLE)	$\Delta H$ (KJ/MOLE)	$\Delta S$ (KJ/MOLE)
Compound-I- $\beta$ - CD	420.9	-14.98	-12.105	0.00965
Compound-II- $\beta$ - CD	603.3	-11.824	-12.010	-0.00625
Compound-III- $\beta$ - CD	231.3	-13.489	-10.36	0.0105

The synthesis of inclusion complexes of compound I (benzylidenamino-1,3,4- thiadiazino [6,5b]indole), compound II(2-[2-nitrobenzylidenamino]-1,3,4-thiadiazino[6,5b]indole) and compound III (2-[2-hydroxybenzylidenamino]-1,3,4-thiadiazino[6,5b]indole) were confirmed

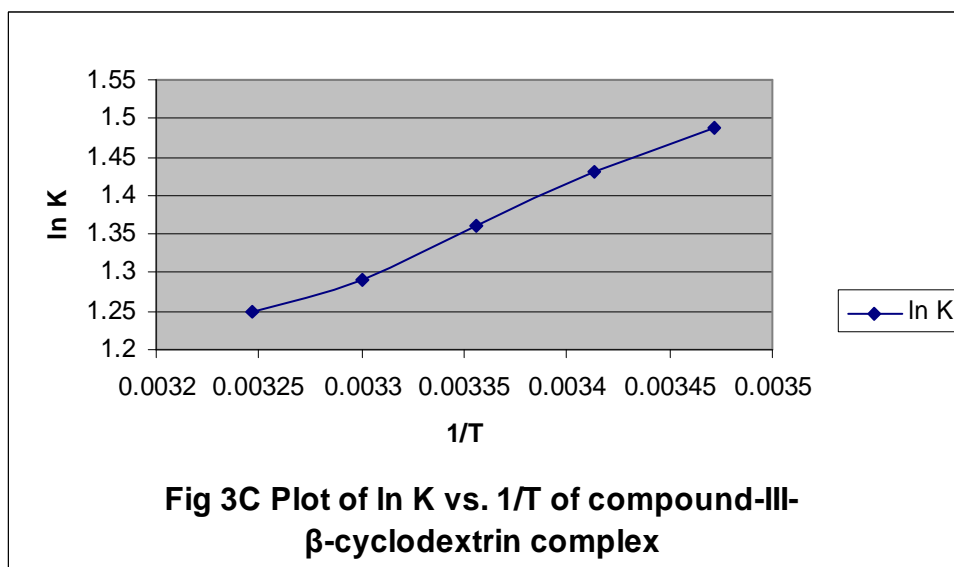
from changes in melting point, colour and spectral characteristics (UV-Vis and IR). The melting point of compound I, II and III are found to be 224°C, 230°C and 239°C but their inclusion complexes have melting points 228°C, 236°C and 246°C (Table-1). The colour of the compound I, II and III are found to be yellow but their inclusion complexes have colours whitish yellow, pale yellow and whitish yellow respectively. The absorption maximum of the compounds I, II and III are found at 3550, 3560 and 3540 Å but their inclusion complexes have absorption maximum at 3542, 3551 and 3530 Å. The higher melting point of inclusion complexes than the compounds is due to the fact that extra amount of thermal energy is required for the latter to bring it out of β-cyclodextrin cavity.











It is quite interesting to note that the absorption maxima undergo a shift towards lower wavelength after the formation of inclusion complex.(Table-I) .This may be attributed to the transference of the compound from a more protic environment to a less protic environment with in the cavity of β- cyclodextrin. This is further supported by IR data The IR stretching frequencies due to different bonds undergo a downward shift towards low energy and the peaks become broader, weaker and smoother. Such changes in spectral characteristics due to inclusion complex formation may be due to the weak interaction like H-bonding, vanderWaal's forces, hydrophobic interactions etc. between the guest compound and the host β- cyclodextrin[16,17]

The phase solubility plots of the compounds in β- cyclodextrin solution are shown in Figure 1A, 1B and 1C. In all the cases, it is seen that there is a linear increase in solubility of these compounds with increasing concentration of β- cyclodextrin.Since the slopes of all the plots are less than unity the stoichiometry of these complexes may be 1:1.[18]

The thermodynamic stability constants ( $K_T$ ) of inclusion complexes were determined by using Benesi-Hilderband relation

$$1/\Delta A = 1/\Delta E + 1/K_T[\text{Guest}]_o.[\beta\text{-CD}]_o$$

Good linear correlations were obtained for a plot of  $1/\Delta A$  verses  $[\beta\text{-CD}]_o$  for compounds. (Figure 2A, 2B and 2C) The values of  $K_T$  for all the complexes were calculated using the relation

$$K_T = \text{Intercept/Slope}$$

The  $K_T$  values of the inclusion complexes of compounds I,II and III with β- cyclodextrin are found to be 420.9,603.3,231.3  $M^{-1}$  respectively(Table-2) The data obtained are within 100 to 1000  $M^{-1}$ (ideal values) indicating appreciable stabilities for the inclusion complexes[19].

The thermodynamic parameters associated with the interaction of the compound with β- cyclodextrin for 1:1 stoichiometry have also been calculated by determining stability constant ( $K$ -values) at different temperatures. The  $K$ - values are to found to decrease with rise in temperature as expected for an exothermic process (deencapsulation)[20,21]. The plots of ln K versus inverse absolute temperature produce linear plots(Figure 3A,3B and 3C). From the slopes of the curves,

van'tHoff's reaction isotherm and van'tHoff equation, the values of  $\Delta G$  (change in free energy),  $\Delta H$  (change in enthalpy) and  $\Delta S$  (change in entropy) have been calculated (Table-2). In Table-2, it is found that  $\Delta G$  values are negative for all the inclusion complexes. These data clearly demonstrates that formation of inclusion complexes of compounds I, II and III with  $\beta$ -cyclodextrin is a spontaneous process. Further it is found that in case of all three inclusion complexes,  $\Delta H$  values are negative (Table-2). The negative value of enthalpy change ( $\Delta H$ ) indicates that all the three inclusion complex formation is exothermic and energy allowed processes. That is, the compounds are getting stabilized within the cavity of  $\beta$ - cyclodextrin by weak intermolecular forces as suggested earlier. It is interesting to note that the entropy change ( $\Delta S$ ) for the inclusion complex-I and III is positive but that of compound -II is negative. The positive value of entropy change further suggests that the formation of inclusion complexes of compound- I and III are entropy allowed. But the smaller negative value of entropy change for the complex of compound-II is due to steric barrier (deactivating nitro group) caused by less free movement by guest molecule within the cavity of host. The study further suggests that the change in entropy destabilizing inclusion complex is compensated by change in enthalpy[22,23]

### CONCLUSION

From the above results and discussion, it is clear that the formation of inclusion complexes of compound-I, II and III is thermodynamically allowed which can be a very good analytical tool for enhancing the bioaccessibility of the drugs. The study further reveals that non-covalent intermolecular forces bind the host  $\beta$ - cyclodextrin and guest molecules. The  $\Delta G$ ,  $\Delta S$  and  $\Delta H$  values support the formation of such complexes.[24]

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