Salicylanilide /Cyclodextrin Inclusion Complex: Preparation, Characterization and Molecular Docking Studies

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Abstract

Interaction between Salicylanilide (SA) with β -Cyclodextrin (β -CD) has been investigated by UV and fluorescence techniques. The red shift in λ_{max} enhanced absorption and emission in β -CD medium confirms the $-\text{NH}-\text{CO}-\text{C}_6\text{H}_5$ aromatic side chain SA is inserted in the β -CD cavity. The stoichiometry and binding constant of 1:1 inclusion complex was calculated using the Benesi-Hildebrand plot derived UV and fluorescence studies and the thermodynamic parameters (ΔG , ΔH and ΔS) of inclusion process were also determined. The results indicated that the inclusion process was an exergonic and spontaneous process. The complex was investigated by FTIR, XRD, Semi empirical and molecular docking methods. The SA: β -CD inclusion complex obtained by molecular docking studies was in good correlation with the results obtained through experimental methods.

Keywords: β -Cyclodextrin; Salicylanilide; Inclusion Complex; Patchdock Server

Introduction

Salicylanilides (SA) is the amide of salicylic acid and aniline. It is classified as both a salicylamide and an anilide. SA is a group of anthelmintics which exert their action by uncoupling mitochondrial reactions which are critical to electron transport and associated phosphorylation in the metabolic system of the parasite. They are effective against cestodes and trematodes but not nematodes. Some are active against *Haemonchus contortus*. Derivatives of salicylanilide have a variety of pharmacological uses. Chlorinated derivatives including niclosamide, oxyclozanide and rafoxanide are used asanthelmintics, especially as flukicides. Brominated derivatives including dibromsalan, metabromsalan and tribromsalan are used as disinfectants with antibacterial and antifungal activities.

CycloDextrins (CDs) are cyclic oligosaccharides obtained from enzymatic hydrolysis of starch. The β -CD is the most abundant natural oligomers and corresponds to the association of seven glucose units with cavity, which exhibits a hydrophobic character whereas the exterior is strongly hydrophilic. Their ability to form host-guest complexes has led to the use of CDs in a

number of fields [1, 2]. CDs have been used in the pharmaceutical industry, as solubilizers, diluents and as tablet ingredients which improve the chemical stability, solubility, bioavailability and pharmacokinetic properties of drugs. In this paper, we report the photo physical and computational studies on the complexation SA and β -CD at different conditions. In addition to the UV and Fluorescence studies, we have utilized the complexation behavior of SA for the stoichiometry and binding constant of SA: β -CD inclusion complex. Further it was also supported by XRD, semiempirical method and molecular docking studies.

Materials and Methods

Materials

Salicylanilide and β -CD were obtained from Aldrich, Hi Media Laboratories and used without further purification. Triply distilled water was used to prepare all solutions and spectro grade solvents were used. Solutions in the pH 7 were prepared by adding the appropriate amount of NaOH and H_3PO_4 . The concentration of β -CD was varied from zero to $1.2 \times 10^{-3} \text{ mol dm}^{-3}$. From the stock solution 2, 4, 6, 8, 10 and $12 \times 10^{-3} \text{ mol dm}^{-3}$ of β -CD were prepared using pH~ 7 buffers. The concentrations of the solutions were of the order $10^{-4} \text{ mol dm}^{-3}$. All experiments were carried out at 30°C. The solid inclusion complex was also prepared by coprecipitation method.

Methods

The pH values were measured using Elico pH meter LI-120. The UV spectra were recorded with Specord 200+ spectrophotometer, Germany. The Fluorescence spectra were recorded using Spectro fluorometer, Perkin Elmer, USA. The IR spectra of all samples were recorded using Alpha-T FTIR Spectrometer (Bruker optics) equipped KBr by using a clean glass pestle and mortar. Powder X-ray diffraction spectra were taken by XPert PRO PANalytical diffractometer. The most probable structure of the SA: β -CD inclusion complex was determined by molecular docking studies using PatchDock server [3].

Results and Discussion

Host–Guest Interaction of SA with β -CD

The absorption spectral data of SA in different concentrations of β -CD recorded in pH~7 are compiled in Table 1. In SA upon increasing the concentration of β -CD a slight red shift is observed in the absorption maxima in the LW and SW absorption bands. No clear isosbestic point is observed in absorption spectrum. The absorption spectra show only very slight change in absorption maxima even in the presence of highest concentration of β -CD used (12×10^{-3} M) in pH~7. This behavior has been attributed to the enhanced dissolution of SA molecules through the hydrophobic interaction between guest molecule (SA) and non-polar cavity of β -CD [4-6] as reported by others also [7, 8]. Since, this indicates the formation of 1:1, host–guest inclusion complex of SA: β -CD.

The binding constant for the formation of SA: β -CD complex has been determined by analyzing the changes in the intensity of absorption maxima with the β -CD concentration. In the case of inclusion complex formed between SA and β -CD, the equilibrium can be written as,

The binding constant 'K' and stoichiometric ratios of the inclusion complex of SA can be determined according to the Benesi–Hildebrand [9] relation assuming the formation of a 1:1 host–guest complex.

$$\frac{1}{A - A_0} = \frac{1}{\Delta \varepsilon} + \frac{1}{K [\text{Salicylanilide}]_0 \Delta \varepsilon [\beta\text{-CD}]_0}$$

Where, A and A_0 is the difference between the absorbance of SA in the presence and absence of β -CD, $\Delta \varepsilon$ is the difference between the molar absorption coefficient of SA and the inclusion complex, $[\text{SA}]_0$ and $[\beta\text{-CD}]_0$ are the initial concentration of SA and β -CD respectively. The plot of $1/A - A_0$ versus $1/[\beta\text{-CD}]$ for SA in pH~7. For pH~7 solutions, a good linear correlation was obtained, confirming the formation of a 1:1 inclusion complex. From the intercept and slope values of this plot, the binding constant 'K' was evaluated. The 'K' value for SA in neutral condition (258.5 M^{-1} at pH~7) at 303K.

The effect of β -CD on the fluorescence spectra of SA (Table 1) is different from absorption spectra and more pronounced than the relative effect on the absorption spectra. In SA, there is no significant change is observed in emission maxima (~528 nm) at pH~7. The emission intensity of SA in pH~7 is increases when the β -CD concentration is increased (Figure 1), whereas the intensity is increased. Figure 1 shows the Benesi–Hildebrand plot of observed changes in the fluorescence intensity with increasing concentration of β -CD. It is seen from this plot that the emission

intensity of SA initially increases with β -CD concentration and then saturates to a limiting value at 0.012M β -CD, indicating the maximum inclusion of SA molecule in the β -CD cavity. The

binding constant for the formation of complex has been

Figure 1: The Fluorescence spectra of Salicylanilide (pH~7) in different β -CD concentrations (mol dm^{-3}): (1) 0.0 M, (2) 0.002 M, (3) 0.004 M, (4) 0.006 M, (5) 0.008 M, (6) 0.010 M and (7) 0.012 M. (Inside- Benesi–Hildebrand plot of $1/F - F_0$ vs. $1/[\beta\text{-CD}]$ for Salicylanilide in pH~7 solution).

determined by analyzing the changes in the intensity of emission maxima with the β -CD concentration using the Benesi–Hildebrand[9] relation assuming the formation of a 1:1 host – guest complex.

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{K [I' - I_0] [\beta\text{-CD}]_0}$$

Where, $[\beta\text{-CD}]_0$ represents the initial concentration of β -CD, " I_0 " and " I " are the fluorescence intensities in the absence and presence β -CD respectively, and I' is the limiting intensity of fluorescence. The 'K' value was estimated from the slope and intercept of the Benesi–Hildebrand plot which shows a good linear correlation supporting the assumption of 1:1, SA: β -CD inclusion complex. The binding constant 'K' value is SA evaluated as 375.14 M^{-1} .

The thermodynamics of inclusion process

The thermodynamic parameters ΔG for the binding of guest molecule to β -CD cavity can be calculated from the binding constant 'K' by using the following equation

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

The thermodynamic parameters ΔG for the binding of guest molecules (SA) to β -CD cavity are given in Table 1. The negative value of ΔG suggests that the inclusion process proceeded spontaneously at 303K. Considering the above discussions, the possible inclusion mechanism is proposed. Naturally, The inclusion complex formation of SA with β -CD, both guest molecules with the $-\text{NH}-\text{CO}-\text{C}_6\text{H}_5$ aromatic side chain and benzene ring of SA inserted in the β -CD cavity as shown in Scheme 1.

Characterization of SA: β -CD complex

The infrared (FTIR) spectra of wave number from 4000 to 400 cm^{-1} of salicylanilide, β -CD and the solid inclusion complex of Salicylanilide with β -CD were registered by FTIR spectrometer. The host molecule (β -CD) reacts with guest molecule (SA) to form host–guest solid complex (SA: β -CD). The solid complex formation can be confirmed by FTIR spectroscopy because, the bands resulting from the included part of the guest molecule are

Table 1: Absorption and fluorescence maxima (nm) of SA (0.001 M) at different concentrations of β -CD in pH~7 solutions.

S. No.	Concentration of β -Cyclodextrin (M)	λ_{max} (nm)	$\log \epsilon$	λ_{flu} (nm)	Flu. Inten
	0 Without β -CD	268	3.55	528	13.403
	0.002	262	3.61	529.5	19.702
	0.004	262	3.63	529.5	23.958
	0.006	262	3.63	529.5	24.18
	0.008	264	3.65	529.5	24.6
	0.01	264	3.66	530	31.821
	0.012	268	3.7	531	33.29
Binding constant (M^{-1})		258.5		375.14	
ΔG (kJ mol^{-1})		-13.99		-14.93	

Scheme 1:

Guest	Bond distance in $^{\circ}\text{A}$		Orientation	inclusion
SA	$\text{H}_{17}-\text{H}_{25}$	11.3	Vertical	partial
	$\text{H}_{23}-\text{H}_{27}$	5.0	Horizontal	

generally shifted or their intensities altered [10]. If β -CD and SA forms a solid inclusion complex, the non-covalent interactions between them such as hydrophobic interactions, vander Waal's interactions and hydrogen bonding lowers the energy of the included part of SA and reduces the absorption intensities of the corresponding bands. We can see that there are apparent differences between the FTIR spectra of β -CD, SA and SA: β -CD solid inclusion complex. The FTIR spectra of SA: β -CD and that of β -CD are alike due to (i) the presence of large number of polar groups such as O-H, C-O, etc., that are responsible for intense absorption bands; (ii) the excess of free (unreacted) β -CD in the SA: β -CD inclusion complex sample. However, the inclusion of SA into the β -CD cavity is evidently confirmed by the bands at 3299, 1501 cm^{-1} , 1334 and 828 cm^{-1} . The IR spectrum of SA examined in KBr pellet, showed one absorption band at 3299 cm^{-1} for N-H stretching with benzene. 1501 cm^{-1} was noted for bending vibration of N-H group attached with benzene ring. The characteristic peak of C-N stretching vibration in aromatic ring appeared at 1334 cm^{-1} . For N-H wagging vibration the peak appeared at 828 cm^{-1} . However, in the IR spectrum of SA: β -CD Inclusion complex, (formed by stirring method) absorption band due to the stretching (at 3299 cm^{-1}) and bending (at 1501 cm^{-1}) vibrations of N-H disappeared, infers that the N-H of SA was entrapped into the β -CD cavity in the inclusion complex. The C-N stretching vibration peak at 1334 cm^{-1} , N-H wagging vibration peak at 828 cm^{-1} shifted in the inclusion complex, indicating the restriction in the vibration of N-H group due to the complete entrapment of N-H containing aromatic ring into β -CD. The aromatic C-H bending vibration at 790 cm^{-1} and C-H out of plane at 895 cm^{-1} disappeared in the inclusion complex. Stretching vibration of C-C in aromatic ring at 1558 cm^{-1} of SA shifted to 1560 cm^{-1} in the SA: β -CD complex. The inclusion complex FTIR spectra peaks are 30-40% weaker than the free SA molecule. The SA: β -CD inclusion complex did not show any new peaks,

indicating that no chemical bonds were created between SA and β -CD in the formed complex which was also confirmed by molecular modeling studies.

Powder X-ray diffraction spectra

The formation of inclusion complex can be confirmed by X-ray diffractometry [11,12]. The Figure 2 shows the powder X-ray diffraction spectra of β -CD, SA and SA: β -CD solid complex. The X-ray spectrum of the inclusion complex shown in Figure 2 b and c was evidently different from that of β -CD Figure 2 a. The difference between the spectra of β -CD and inclusion complex is due to the interaction of β -CD with SA.

Semi empirical quantum mechanical calculations

The internal diameter of the β -CD is approximately 6.5 $\text{\AA}$ and its height is 7.8 $\text{\AA}$ (Scheme 1). Considering the shape and dimensions of β -CD, SA cannot be completely embedded in the

Figure 2: XRD pattern of (a) β -CD, (b) Salicylanilide and (c) Salicylanilide: β -CD solid complex.

Figure 3: Ball and stick representation of (a) β -CD, (b) SA, and (c) SA: β -CD 1:1 inclusion complex. oxygen atoms are shown as red balls, carbon atoms as golden balls and sticks, nitrogen atoms as blue balls and hydrogen atoms are shown in grey balls and sticks.

β -CD cavity. The ground state of SA molecule was optimized using AM1 method. SA the vertical distance between H_{17} - H_{25} is 11.3 Å and this is higher than the height of β -CD. The horizontal distance between H_{23} - H_{27} is 5 Å and is less than the internal diameter of β -CD. Since, the height of SA is higher than that of upper-lower rim of β -CD, the -NH-CO- C_6H_5 aromatic side chain and benzene ring of SA insertion in the β -CD cavity is possible as shown in Scheme 1.

Molecular docking study of inclusion process

The 3D structure of β -CD and SA obtained from crystallographic databases are shown in Figure 3. The guest molecule, SA was docked into the cavity of β -CD using PatchDock server. The PatchDock server gave several possible docked models for the most probable structure based on the energetic parameters; geometric shape complementarity score [13], approximate interface area size and atomic contact energy [14] of the SA: β -CD inclusion complex. The docked SA: β -CD model (Figure 3c) with the highest geometric shape complementarity score 3212 approximate interface area size of the complex 381.90 Å² and atomic contact energy -248.24 kcal/mol was the highly probable and energetically favourable model.

Conclusions

In summary, the inclusion complex with 1:1 molar ratio was formed between β -CD and SA. The -NH-CO- C_6H_5 aromatic side chain and part of benzene ring of SA was inserted in the β -CD. The inclusion complex formation of SA with β -CD, the guest molecules with the -NH-CO- C_6H_5 aromatic side chain and benzene ring of SA inserted in the β -CD. Thermodynamic parameter values show the inclusion processes are spontaneous. UV, Fluorescence, XRD, semi empirical and molecular docking results confirms the formation of SA: β -CD inclusion complex. The inclusion complex

formation which was also confirmed by molecular docking studies.

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