BETA-CYCLODEXTRIN ENCAPSULATED INCLUSION COMPLEX OF ISATIN PHENYLHYDRAZONE: SPECTRO SCOPIC, STOICHIOMETRIC, THERMODYNAMIC, AND BIOLOGICAL PROFILES.

Pankaj Meshram[1]*, Varsha Manvatkar[2], Prachi khobragade[3], Rajendra Dongre[4]

Article History: **Received: 20.05.2023** **Revised: 28.06.2023** **Accepted: 20.07.2023**

Abstract:
Isatin-derived Schiff's base or hydrazones, insoluble in polar medium, have quite a meagre pharmacological profile; however, their innate bio-accessibility gets augmented through host-guest inclusion complexation. This research focuses on the preparation, characterization, and biological evaluation of a poorly water-soluble guest molecule, Isatin phenylhydrazone (IPH), chemically named *3-(2-phenylhydrazone) indolin-2-one.* When the synthesized guest was trapped inside the hydrophobic cavity of host, β-cyclodextrin (β-CD), the inclusion complex (β-CD/IPH) with enhanced bio-assessment profiles was formed. The guest and its inclusion formulations were characterised by reliable spectroscopic techniques like ^{1}H-NMR, UV-Vis, and FTIR spectroscopy. Additionally, the Jobs technique of continuous variation was used to estimate the stoichiometry ratio, which was found to be 1:1 for the β-CD/IPH inclusion complex. A modified Benesi-Hildebrand equation was used to calculate the stability constant for range of temperature. Calculations were made for the inclusion complex's thermodynamic characteristics, including the changes in free energy (ΔG^{o}), enthalpy (ΔH^{o}), and entropy (ΔS^{o}), and it was found that the effective complex formation was spontaneous and exothermic. The antimicrobial profile of guest and host-guest complex was studied by means of the agar-well diffusion method against three pathogenic bacteria, including *Escherichia coli, Staphylococcus aureus, Proteus vulgaris,* and a fungus, *Candida albicans,* using DMSO as a control. Antimicrobial study reports of inclusion assembly *(β-CD /IPH)* were found to be enhanced compared to the mere guest *(IPH)* moiety.

Keywords: Isatin Phenylhydrazone, β-Cyclodextrin, inclusion complex, spectroscopy, thermodynamic parameters, antimicrobial profile.

[1]*,[2,3,4] PGTD chemistry, R.T.M. Nagpur university, Nagpur (M.S), India
[1]*Shri Lemdeo Patil Mahavidyalaya, Mandhal, Nagpur-441210 (M.S.), India
[2]G. D. M. Arts, K. R. N. Commerce and M. D. Science College, Jamner,Jalgaon, M.S.424206, India

***Corresponding Author:** Pankaj Meshram
*PGTD chemistry, R.T.M. Nagpur university, Nagpur (M.S), India
*Shri Lemdeo Patil Mahavidyalaya, Mandhal, Nagpur-441210 (M.S.), India Email: punkaj99@gmail.com

DOI: - 10.48047/ecb/2023.12.si10.00171

1. Introdution

Cyclodextrins are macrocyclic oligomers made up of D-glucopyranoside and exist as six (α-CD), seven (β-CD), and eight-member rings (γ-CD) connected mutually via α-(1, 4) connections [1–3]. Fig.1a. A hollow truncated cone structure of CDs emerges from the circular arrangement of D-glucose with primary hydroxyl groups on one rim (the primary face) and secondary hydroxyl groups on the other (the secondary face) [4]. fig.1b. These morphological features of CD moiety impart numerous utilities, including those in the food industry [5], textiles [6], wastewater treatment [7], the agriculture sector [8], the chemical industry [9], drug delivery systems [10] and pharmaceutical formulation [11] etc. The supramolecular host, CDs, generates non-covalent host-guest interactions with small organic molecules like Isatin derivatives [12]. The interactions are mainly weak forces like electrostatic, van der Waal, hydrogen bonding, and hydrophobic interactions that exist inside the cavity [13]. Such host-guest interactions of CDs with organic molecules have been found to relieve certain conformational strains and liberate innate strain energy that existed prior to their interaction [14, 15]. Among all the CDs, the most commonly used host in pharmaceutical formulations is β-cyclodextrin which possesses natural qualities such as complementary cavity size [16], bio-degradability [17], non-toxicity, and economic price [18].

Indole-based molecules like isatin derivatives have many uses in pharmacology, such as anti-oxidant [19], anti-diabetic [20], anti-microbial [21], anti-cancer [22], anti-glycation [23], anti-plasmodial, anti-tubercular [24], and anti-convulsant [25]. However, their poor solubility and bioavailability can limit their effectiveness as therapeutic agents. The inclusion complex formation between isatin derivatives and β-cyclodextrin has been studied recently due to its potential applications in drug delivery and pharmaceutical formulations. The hydrophobic cavity in β–cyclodextrin encapsulates the hydrophobic portion of the isatin derivative and yields inclusion complex with improved solubility, stability, and bioassay. The driving force in this association is the non-covalent interactions between the guest, isatin derivative, and the host, β–cyclodextrin. The complexation with β-cyclodextrin offers a potential strategy for enhancing the therapeutic potential of isatin derivatives and overcoming their limitations in pharmaceutical applications [26]. Overall, the inclusion of isatin-derived compounds within β-cyclodextrin cavity provides a promising approach to improving the pharmacological profile of these bioactive compounds. There is hardly any research to explain how cyclodextrin complexation affects the physicochemical and antimicrobial properties of isatin derivatives. The purpose of the current study was to synthesise and characterise isatin phenylhydrazone that is formed by condensing isatin with phenyl hydrazine as amine counterpart, and the resulting IPH (fig. 1c) was encapsulated within β-Cyclodextrin's cavity to yield inclusion complex (β-CD/IPH). The thermodynamic and antimicrobial properties of IPH and its inclusion complex have been studied.

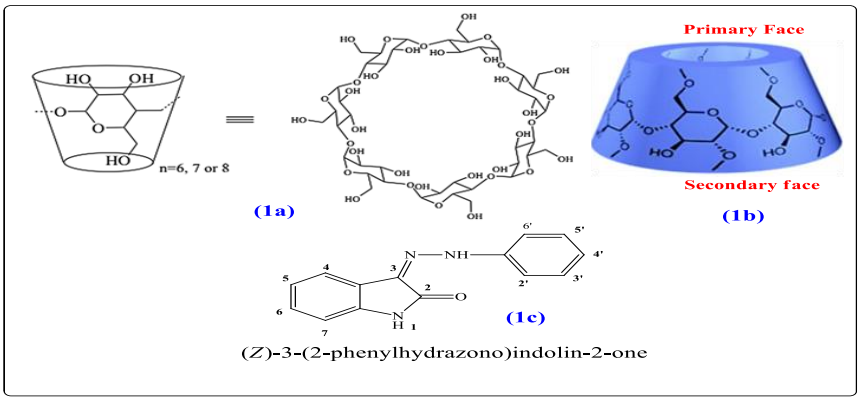

Fig. (1a): Morphology of Cyclodextrins (number of D-Glucopyranose rings in α-CD is 6, in β-CD is 7, and in γ-CD is 8] **(1b)** Primary and secondary faces of β-CD **(1c)** The structure of IPH is named 3-(2-phenylhydrazono)indolin-2-one.

2. Materials and methods:

The analytical reagent grade Chemicals like Isatin, Phenyl hydrazine, DMSO, β-Cyclodextrin etc. were acquire commercially and utilized without further purification. Distilled water was prepared in our laboratory. The KBr disc method was utilised to get FT-IR spectra in KBr in the 400–4000 cm^{-1} range using a Shimadzu Corp. 03093 Fourier transform spectrophotometer. UV spectra have been obtained in the 200–780 nm

regions using a UV-2600 Series double-beam spectrophotometer. NMR spectra in $CDCl_3$/DMSO-d_6 were recorded using the BRUKER Avance Neo (1H NMR, 500 MHz) spectrophotometer. Chemical shift (δ) values are expressed in ppm.

2.1. Stoichiometry of host-guest complex.

The stoichiometry of the host-guest inclusion complex has been determined using Job's method of continuous variation [27]. IPH mole fractions between 0.0 and 1 mmol were used to prepared IPH and β-CD solutions. To create Job's plots, we plotted the absorbance difference between the IPH with and without β-CD (ΔA) multiplied by the mole fraction (R) versus the mole fraction (R). Where R = [IPH]/[[IPH]+ [β-CD]] [28, 29]. Each solution's absorbance values were calculated at a maximum wavelength of 402 nm. The stoichiometry of the inclusion complex is determined by the value of R at the maximums of the Jobs plot.

2.2. Determination of stability constant

The concentration of β-CD was adjusted from 3.0×10^{-5} to 7.0×10^{-5} mol L^{-1}, while the concentration of IPH remained fixed at 5.0×10^{-5} mol L^{-1} in order to calculate the stability constant of the IPH/ β-CD inclusion complex. At 402 nm, the absorbance of each system was measured in comparison to a reagent blank that had been made with the same reagent concentration but without IPH. The UV–Vis absorbances of the aqueous solution at these concentrations were fit to the modified Benesi–Hildebrand's equation. The best fits were obtained for β-CD/IPH inclusion complex. [30, 31].

$$\frac{\mathbf{1}}{\mathbf{\Delta A}} = \frac{\mathbf{1}}{\Delta\epsilon} + \frac{\mathbf{1}}{\boldsymbol{Ks}[\boldsymbol{IPH}]\boldsymbol{o}\Delta\epsilon}\cdot\frac{\mathbf{1}}{[\boldsymbol{\beta-CD}]\mathbf{o}} \qquad (1)$$

Where ΔA is the difference between IPH's absorbance at λmax with and without β-CD, Δε is the difference in the molar absorptivities between free IPH and β-CD/IPH inclusion complex, [IPH]o is the total IPH concentration, and [β-CD]o is the concentration of free β-CD.

Using the Benesi-Hilderband's relation (1), the stability constants (Ks) of the host-guest complex at different temperatures (298.15, 303.15, 308.15, and 313.15 K) were determined using a double reciprocal plot, 1/ΔA vs 1/[β-CD].

2.3. Synthesis of Isatin phenylhydrazone [IPH]:

In 50 mL of warm ethanol, equimolar amounts of Isatin and phenyl hydrazine were dissolved. The content was refluxed for 2 hours. The crystalline product was obtained by filtering, vacuum dried, and recrystallization from ethanol after being allowed to stand for about 24 hours at room temperature (r.t.) [32]. The synthesis of Isatin Phenylhydrazone (IPH) is shown in Scheme 1. (Fig 2.)

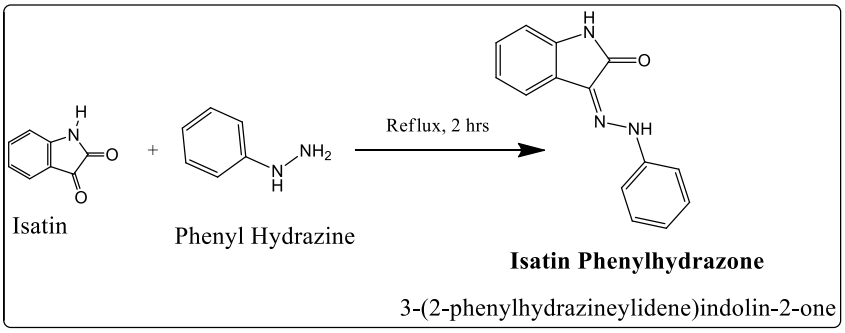

Fig. 2. Scheme 1: Synthesis of Isatin Phenylhydrazone (IPH)

2.4. Synthesis of the Inclusion Complex:

A co-precipitation approach was employed in synthesizing the inclusion complex (1:1 molar ratio). 1 mmol of host: β-CD was dissolved in 20 mL of de-ionized hot water, and then 1 mmol of guest: IPH was dissolved in 20 mL of hot ethanol and added to the aforementioned solution. Then, at room temperature, this solution was agitated for 2 hours on a Remi-magnetic stirrer. To collect the precipitated complex, this solution was filtered using Whatman No. 1 filter paper. The precipitated complex was then washed with a small amount of ethanol and water separately to get rid of any remaining unreacted reactants (IPH and β-CD). For further study, the inclusion complex was collected, dried, and stored in airtight desiccator [33].

2.5. Antimicrobial profile

The synthesised bioactive guest and the associated inclusion complex were subjected to the agar-well diffusion technique to evaluate their antibacterial

profiles using DMSO as a control [34]. This bioassay study was carried out using three bacterial strains, namely E. coli, S. aureus, and P. vulgaris, and a fungus strain, Candida albicans. The agar plate surface was inoculated under aseptic environment by covering the whole agar surface with a volume of the microbial inoculum. Using a sterile cork borer, a hole of 6-8 mm in diameter was punched into the agar plate. The bioactive samples under study were serially diluted in DMSO solvent to make concentrations of 100, 50, 25, and 12.5 ug/ml, and then added to the agar plate's well. These agar plates were incubated for 24 hours at 37 °C. The microbial strain's growth was found to be inhibited by the antimicrobial agent utilized in this bioassay investigation, and the diameters of the zones of inhibition were determined in mm.

3. Results & Discussion:

3.1. Synthesis of IPH and β-CD/IPH:

The structural elucidation of synthesized IPH and its corresponding inclusion complex was carried out by spectral analysis like IR, ^{1}H-NMR, and UV-visible. The spectral data of IPH were in agreement with the assigned structure (Fig. 1c). The melting point of inclusion complex was found to be higher than that of corresponding guest species, and these preliminary observations directed the formation of inclusion complex.

3.1.1. 3-(2-phenylhydrazono)-indolin-2-one (IPH) [35]

Colour: orange, m.p. - 210 °C

1H-NMR ($CDCl_3$, δ in ppm): 6.89–7.12 (m, 3H,-5, 6,7), 7.21–7.39 (m, 5H- 2', 3', 4', 5', 6'), 7.6608–7.6456 (d, 1H, 4), 7.7844 (s, 1H, NH), 12.71 (s, 1H, N=NH); **FT-IR (υ in cm^{-1}):** 3125 (enolic O-H), 1683 (C=O), 1555 (C=N), 1464–1492 (C=C), 1230 (N-H), 741, 686 (Ar-H); **UV-visible (λmax in nm):** 402nm

3.1.2. β-CD/IPH inclusion complex [36]:

Colour: orange-yellow, **m.p. : 225 °C**

1H-NMR (DMSO-d6, δ in ppm): (s, 1H, -N=NH) δ=12.7451, (s, 1H, N-H) δ=11.0166, (m, Ar-H, 9H) δ=6.9199-7.5607, (β -CD H) δ=3.5565-5.06875; FT-**IR (υ in cm^{-1}):** 3260 (enolic OH) 1684 (C=O), 1555 (C=N), 1449 (C=C), 1243 (N-H), 746-687 (Ar-H); **Uv-Visible (λmax in nm):** 410nm

3.2. FTIR Spectroscopy:

Fourier transformation infrared (FTIR) is an important spectroscopic technique used to find fingerprint regions and characteristic IR peaks of guest and host (separately) as well as host-guest molecule in corresponding inclusion complex [37,38,39]. When the host encloses the guests in their own hydrophobic cavities, the guests' bending and stretching vibration peaks weaken or disappeared which demonstrated by shift in the FTIR signals and intensity variations. A broad peak was found to appear at 3281 cm^{-1}, being related to O-H stretching, 2925 cm^{-1} for C-H stretching, and 1022 cm^{-1} for the symmetric C-O-C stretching frequencies observed in the host: β-Cyclodextrin (Fig. 3a.) [33]. The characteristic functionality of IPH was observed at 3125 cm^{-1} for O-H (enolic), 1552 cm^{-1} for C=N, 1683 cm^{-1} for C=O, and 1230 cm^{-1} for the ring N-H vibrational frequencies (Fig. 3b). The FTIR spectra of the inclusion complex were found to be comparable to host β-CD due to non-covalent bonding. Overall, the FTIR spectrum indicated a few modifications in the fundamental vibration frequencies of the guest IPH and host β-CD in the complex (Fig. 3c). The peaks for C=O, C=N and ring N-H of guest after encapsulation shifted to 1684 cm^{-1}, 1555 cm^{-1} and 1243 cm^{-1} respectively while the host β-CD shows signals at 3260 cm^{-1} and 1024 cm^{-1} for O-H and C-O-C functionalities respectively. Changes in the FTIR spectral characteristics can be linked to weak interactions between the host and guest, like hydrogen bonding, Van der Waal interactions, and hydrophobic interactions.

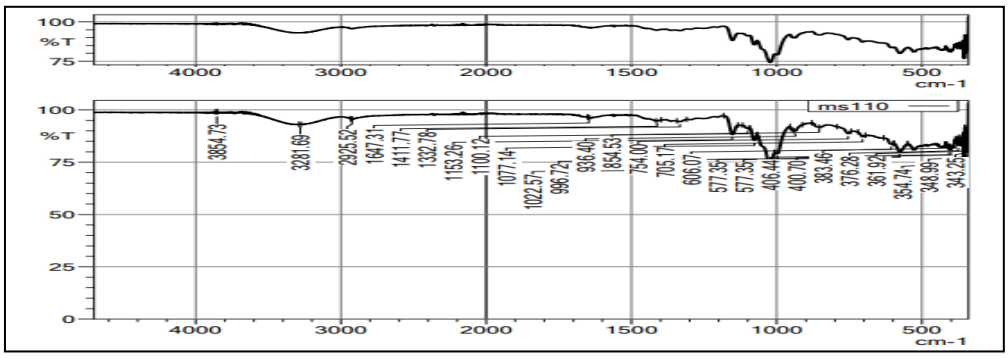

Fig.3a. IR spectra of β –Cyclodextrin

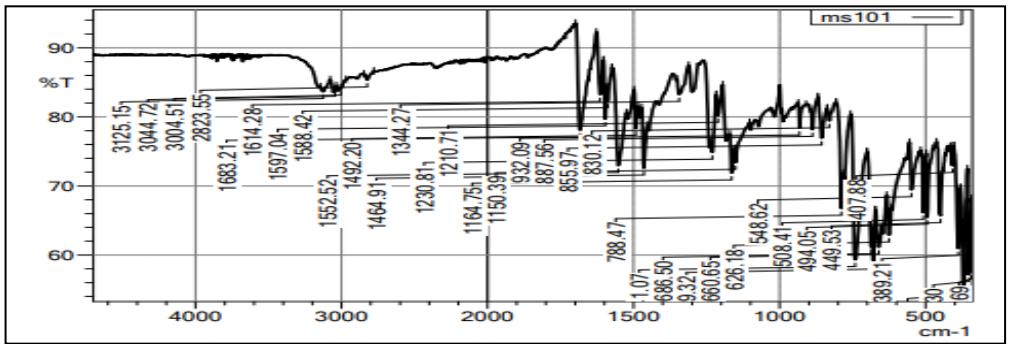

Fig. 3b. FTIR spectra of IPH

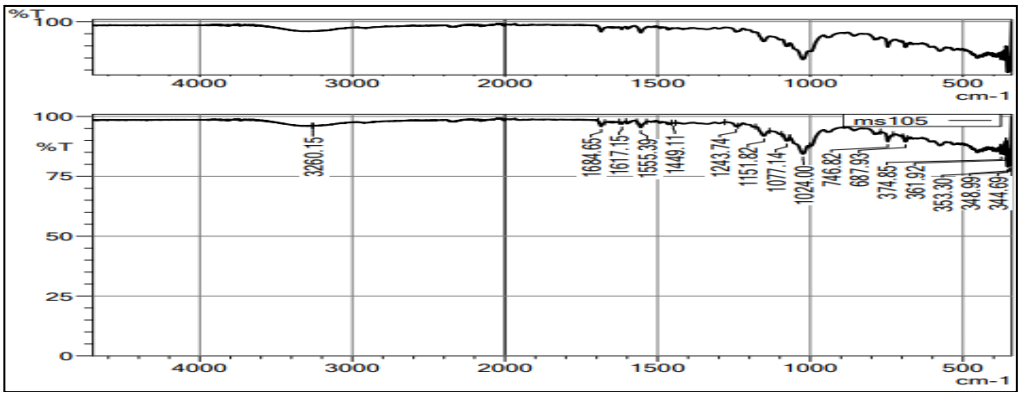

Fig. 3c: FTIR spectra of β-CD/IPH inclusion complex

3.3. [1]H-NMR spectra:

The [1]H-NMR study demonstrates that complexation had an impact on the chemical shifts of particular guest and host [40]. These adjustments helped to clarify the host-guest relationship between IPH and β-CD. As the host and guest do not form any chemical bonds, the interaction primarily relies on the intermolecular forces that exist between the combining molecules. In accordance with the development of the inclusion complex, the [1]H-NMR spectra of the IPH, β-CD, and β-CD/IPH in DMSO-d6 (Figs. 4a, 4b, and 4c) shows the anticipated proton signals of the guest molecule and β-cyclodextrin. The chemical shift changes of the guest molecules compared to the inclusion form were also studied throughout the formation of the inclusion complex. The ring NH, -N=NH proton of IPH underwent the most substantial modifications. Therefore, it was logical to suppose that guest molecule actively engaged in hydrogen bonding with β-CD. The characteristic chemical shift values of β-CD protons in inclusion complexes were found to be modified from the δ=3.2960-5.7207 to δ=3.5565-5.06875.

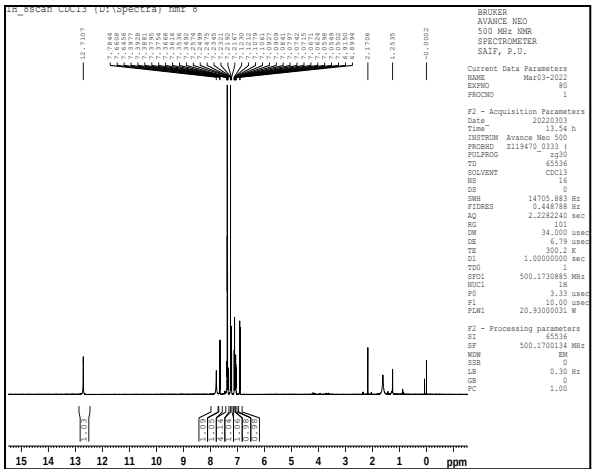

Fig. 4a. 1H-NMR spectra of IPH

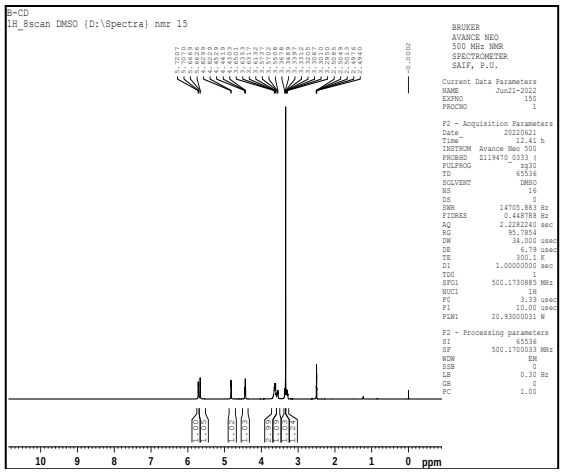

Fig. 4b. 1H-NMR spectra of β-CD

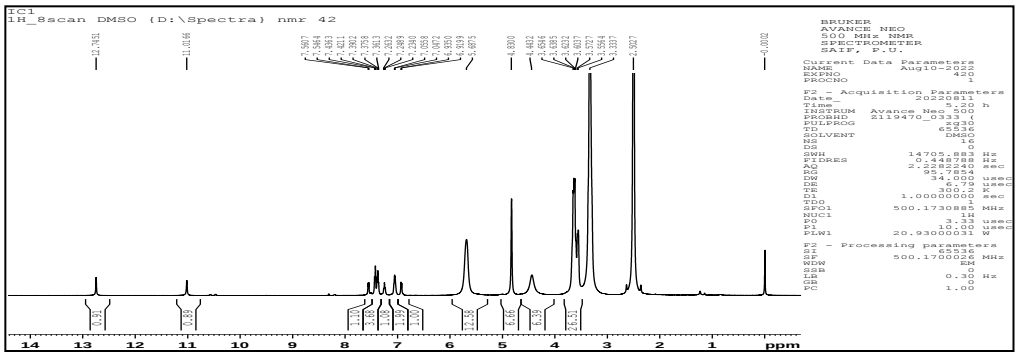

Fig. 4c. 1H-NMR spectra of β-CD/IPH inclusion complex

3.4. Absorption spectra:

The formation of inclusion complexes is also ascertained from the shifting of λmax values of free guest and inclusion complexes [41]. IPH shows λmax at 402 nm, while upon complexation, the bathochromic effect is observed and the maximum wavelength shifts to 410nm. The alteration in λmax might be due to the weak forces that exist between host and guest.

3.4. Stoichiomtery:

The stoichiometry of the host guest association plays an important role when determining the inclusion complex's stability constant. The stoichiometry of the inclusion complex is determined by the value of mole ration (R) at the highest deviation of ΔA×R vs. R plot (Jobs plot). The ratio of guest to host is 1:2 if R = 0.33; 1:1 if R = 0.5; 2:1 if R = 0.66, etc. The maxima for the plot in the current study were obtained at R = 0.5, suggesting a 1:1 stoichiometry for the host-guest inclusion complexes Fig. 5a [42].

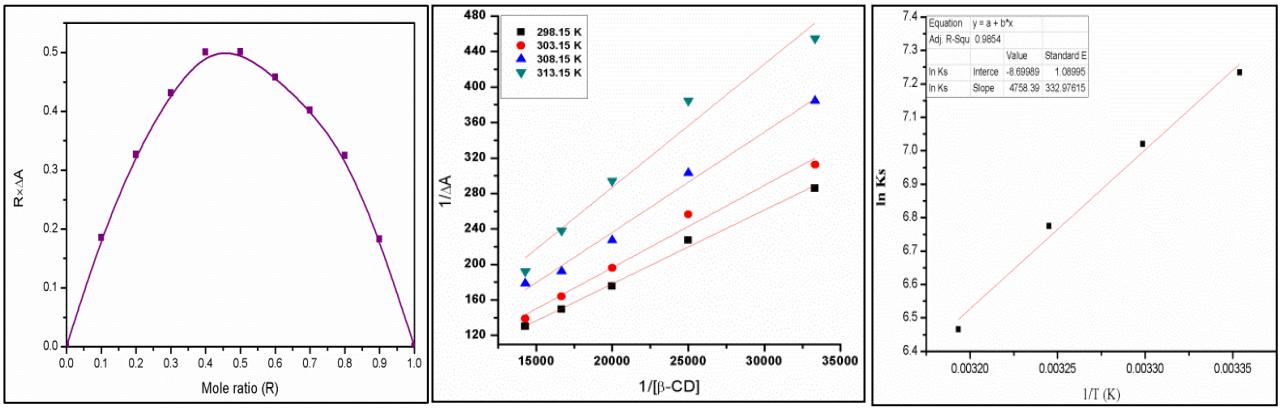

Fig. 5a **Fig. 5b** **Fig. 5c**

Fig.: **(5a)** Job's plot of IPH-β-CD systems at λmax=402nm, mole ratio R=[IPH]/[[IPH]+[β-CD]], ΔA=absorbance difference of the IPH without and with β-CD; **[5b)** Variation of 1/ΔA against 1/[β-CD] at 298.15, 303.15, 308.15, and 313.15 K; **(5c)** Variation of ln Ks against 1/T

3.5. Stability constant (Ks) and allied thermodynamic parameters (ΔG°, ΔH° and ΔS°):

The stability constant (Ks) is an important property in the inclusion phenomenon that measures the effectiveness of host-guest complexation [43]. The stability constants (Ks) can be calculated using the changes in absorbance (ΔA) of guest molecules against the concentration of β-cyclodextrin at different temperature [44]. The absorbance data collected for various concentrations of β-CD demonstrated that when the concentration of β-CD rise, the guest molecule's aqueous solubility was observed to be gradually raised.

From the plots in Fig. 5b, we got a good linear correlation, which is evident from correlation coefficient values that are close to unity, which confirmed the stoichiometry of the complex was 1:1. The stability constant (Ks) for studied temperature range was determined by equation 2. (Table 1).

$$\mathbf{Ks} = \frac{\boldsymbol{Intercept}}{\boldsymbol{Slope}} \qquad \textbf{(2)}$$

The temperature markedly affects the host-guest association, as the study suggests the decapsulation of guests from the β-CD cavity at

higher temperatures. Stability constant values were found to decrease with increasing temperature. The thermodynamic parameters such as free energy change (ΔG), enthalpy (ΔH), and entropy change (ΔS) associated with the inclusion of complex formation at studied temperature range were determined using calculated Ks values [45, 46, 47]. The relation (3) was used to calculate the change in free energy.

$$\Delta G = -RTlnk \quad (3)$$

Using Vant't Hoff equation 4, enthalpy and entropy changes have been determined.

$$lnK = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (4)$$

R is the gas constant (8.314 J K-1 mol−1), and T is the absolute temperature.

From Vant Hoff's equation 4, the plot between ln Ks and 1/T shows a linear relationship (fig. 5c) [48, 49]. The interaction between IPH and β-CD was an exothermic process that occurred spontaneously within the investigated temperature range. The thermodynamic parameter changes specify that the host-guest association is mainly driven by weak forces such as Vander Waal, hydrogen bonding, etc. between the interacting species. When compared to the guest alone, the inclusion complex's rotational and vibrational degrees of freedom are assumed to be lower [50]. The results are summarized in Table 1.

Table 1: Stability constant (Ks) and thermodynamic parameters of the β-CD/ IPH inclusion complex

T (K)	K_s (M^{-1})	ΔG^o (kJ / mol)	ΔH^o (kJ / mol)	ΔS^o (kJ / mol k^{-1})
298.15	1385.9	-17.935		
303.15	1118.52	-17.695	-39.561	-0.072
308.15	875.39	-17.359		
313.15	642.4	-16.835		

3.6. Antimicrobial study:

Compared to the free guest, the inclusion complex had a better antimicrobial profile against the strains of E. coli, S. aureus, P. vulgaris, and C. albicans that were studied. The guest IPH and its inclusion complex, β-Cd/IPH, had a unique efficiency against Proteus vulgaris, with zones of inhibition at 100 ug/ml that were 17mm and 24 mm, respectively. When IPH complexes with β-CD, the guest's antifungal efficacy against Candida albicans increases. The solubility, bioavailability, and bioaccessibility of the included guest that are induced following encapsulation within the hydrophobic cavity of the host are responsible for the inclusion complex's better antimicrobial characteristics [51]. The inclusion product increased lipophilicity of IPH, which decreased the permeability of the cells and interfered with normal cell functions. Figures 6a–d represents the graphical summary of the findings.

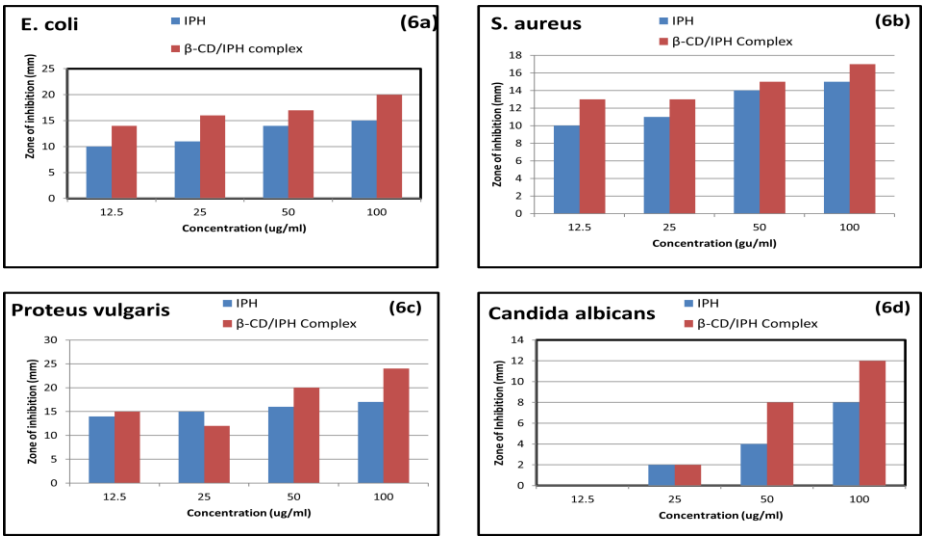

Fig. 6: Comparative antimicrobial assay of guest [IPH] and inclusion complex [β-CD/IPH] in terms of zone of inhibition (in Mm) against Escherichia coli (6a), Staphylococcus aureus (6b), Proteus vulgaris (6c), and Candida albicans (6d) at 12.5, 25, 50, and 100 ug/ml concentrations of guest and inclusion complex separately.

4. Conclusion:

In this study, the feasibility of employing β-CD to encapsulate Isatin phenylhydrazone via inclusion complexation has been demonstrated. UV-visible, FTIR, 1H-NMR, and stability Investigations showed that the guest was effectively encapsulated inside the hydrophobic cavity of β-CD. The 1:1 stoichiometry ratio obtained by Jobs method of host-guest interaction was found to be exothermic and spontaneous by thermodynamic analysis. The study of this complex reveals details on the non-covalent intermolecular forces that bind the molecules which make up the "host-guest" pair. We also investigated the antimicrobial activity of the synthesized compounds. When compared to guest, the complex was found to be more active against bacterial species *(such as E. coli, S. aureus, and Proteus vulgaris)* and fungal species *(Candida albicans).* Cyclodextrins are currently commonly used for medication stabilization by delivering highly selective and non-toxic possibilities. This complexation approach open up new avenue for the enhancement of anti-microbial profile of bioactive compounds. Given the ease of preparation and environmental friendliness of inclusion complexes, it is an appealing strategy for creating new Isatin precursor prototypes with excellent water solubility and bioavailability for potential medical applications.

Acknowledgement:

We thankfully acknowledge the late Prof. Jyotsna S. Meshram, the former head of the PGTD at R. T. M. Nagpur University, Nagpur, for her enormous support and supervision. The authors acknowledge the Head, PGTD of Chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra (India), for the immense support, and SAIF Chandigarh University, Punjab, for recording 1H-NMR spectra.

References:

1. L. Almagro and M. Á. Pedreño, “Use of cyclodextrins to improve the production of plant bioactive compounds,” Phytochemistry Reviews, vol. 19, no. 4. Springer Science and Business Media B.V., pp. 1061–1080, Aug. 01, 2020. doi: 10.1007/s11101-020-09704-6.
2. A. H. Mazurek and Ł. Szeleszczuk, “A Review of Applications of Solid-State Nuclear Magnetic Resonance [ssNMR] for the Analysis of Cyclodextrin-Including Systems,” International Journal of Molecular Sciences, vol. 24, no. 4. MDPI, Feb. 01, 2023. doi: 10.3390/ijms24043648.
3. Z. Li et al., “γ-Cyclodextrin: A review on enzymatic production and applications,” Applied Microbiology and Biotechnology, vol. 77, no. 2. pp. 245–255, Nov. 2007. doi: 10.1007/s00253-007-1166-7.
4. A. A. Sandilya, U. Natarajan, and M. H. Priya, “Molecular View into the Cyclodextrin Cavity: Structure and Hydration,” ACS Omega, vol. 5, no. 40, pp. 25655–25667, Oct. 2020, doi: 10.1021/acsomega.0c02760.
5. Y. Liu, Y. Chen, X. Gao, J. Fu, and L. Hu, “Application of cyclodextrin in food industry,” Critical Reviews in Food Science and Nutrition, vol. 62, no. 10. Taylor and Francis Ltd., pp. 2627–2640, 2022. doi: 10.1080/10408398.2020.1856035.
6. B. G. Poulson et al., “Cyclodextrins: Structural, Chemical, and Physical Properties, and Applications,” Polysaccharides, vol. 3, no. 1, pp. 1–31, Dec. 2021, doi: 10.3390/polysaccharides3010001.
7. M. Y. Xu, H. L. Jiang, Z. W. Xie, Z. T. Li, D. Xu, and F. A. He, “Highly efficient selective adsorption of anionic dyes by modified β-cyclodextrin polymers,” J Taiwan Inst Chem Eng, vol. 108, pp. 114–128, Mar. 2020, doi: 10.1016/j.jtice.2020.01.005.
8. B. Cheirsilp and J. Rakmai, “Inclusion complex formation of cyclodextrin with its guest and their applications, Biology, Engineering and Medicine. 2[2017].” 2017.
9. E. M. M. Del Valle, “Cyclodextrins and their uses: A review,” Process Biochemistry, vol. 39, no. 9. pp. 1033–1046, May 31, 2004. doi: 10.1016/S0032-9592[03]00258-9.
10. D. H. Kim, S. E. Lee, Y. C. Pyo, P. Tran, and J. S. Park, “Solubility enhancement and application of cyclodextrins in local drug delivery,” Journal of Pharmaceutical Investigation, vol. 50, no. 1. Springer, pp. 17–27, Jan. 01, 2020. doi: 10.1007/s40005-019-00434-2.
11. S. S. Jambhekar and P. Breen, “Cyclodextrins in pharmaceutical formulations I: Structure and physicochemical properties, formation of complexes, and types of complex,” Drug Discovery Today, vol. 21, no. 2. Elsevier Ltd, pp. 356–362, 2016. doi: 10.1016/j.drudis.2015.11.017.
12. R. K. Mahato, A. Debnath, A. Das, D. Sarkar, S. Bhattacharyya, and B. Biswas, “Supra-molecular encapsulation of nanocrystalline Schiff bases into β-cyclodextrin for multifold enrichment of bio-potency,” Carbohydr Polym, vol. 291, p. 119614, 2022, doi: https://doi.org/10.1016/j.carbpol.2022.119614.

13. J. U. Lee, S. S. Lee, S. Lee, and H. Bin Oh, "Noncovalent complexes of cyclodextrin with small organic molecules: Applications and insights into host–guest interactions in the gas phase and condensed phase," Molecules, vol. 25, no. 18. MDPI AG, Sep. 01, 2020. doi: 10.3390/molecules25184048.
14. F. Ortega-Caballero, C. Rousseau, B. Christensen, T. E. Petersen, and M. Bols, "Remarkable Supramolecular Catalysis of Glycoside Hydrolysis by a Cyclodextrin Cyanohydrin," J Am Chem Soc, vol. 127, no. 10, pp. 3238–3239, Mar. 2005, doi: 10.1021/ja042678a.
15. F. Ortega-Caballero, J. Bjerre, L. S. Laustsen, and M. Bols, "Four Orders of Magnitude Rate Increase in Artificial Enzyme-Catalyzed Aryl Glycoside Hydrolysis," J Org Chem, vol. 70, no. 18, pp. 7217–7226, Sep. 2005, doi: 10.1021/jo050861w.
16. K. M. Sahu, S. Patra, and S. K. Swain, "Host-guest drug delivery by β-cyclodextrin assisted polysaccharide vehicles: A review," Int J Biol Macromol, vol. 240, p. 124338, 2023, doi: https://doi.org/10.1016/j.ijbiomac.2023.124338.
17. X. Zhou et al., "Biodegradable β-Cyclodextrin Conjugated Gelatin Methacryloyl Microneedle for Delivery of Water-Insoluble Drug," Adv Healthc Mater, vol. 9, no. 11, Jun. 2020, doi: 10.1002/adhm.202000527.
18. S. V Kurkov and T. Loftsson, "Cyclodextrins," Int J Pharm, vol. 453, no. 1, pp. 167–180, 2013, doi: https://doi.org/10.1016/j.ijpharm.2012.06.055.
19. J. Haribabu, G. R. Subhashree, S. Saranya, K. Gomathi, R. Karvembu, and D. Gayathri, "Isatin based thiosemicarbazone derivatives as potential bioactive agents: Anti-oxidant and molecular docking studies," J Mol Struct, vol. 1110, pp. 185–195, 2016, doi: https://doi.org/10.1016/j.molstruc.2016.01.044.
20. A. S. Hassan, N. M. Morsy, W. M. Aboulthana, and A. Ragab, "Exploring novel derivatives of Isatin-based Schiff bases as multi-target agents: design, synthesis, in vitro biological evaluation, and in silico ADMET analysis with molecular modeling simulations," RSC Adv, vol. 13, no. 14, pp. 9281–9303, Mar. 2023, doi: 10.1039/d3ra00297g.
21. H. Guo, "Isatin derivatives and their anti-bacterial activities," European Journal of Medicinal Chemistry, vol. 164. Elsevier Masson SAS, pp. 678–688, Feb. 15, 2019. doi: 10.1016/j.ejmech.2018.12.017.
22. Y. Hou, C. Shang, H. Wang, and J. Yun, "Isatin–azole hybrids and their anticancer activities," Archiv der Pharmazie, vol. 353, no. 1. Wiley-VCH Verlag, Jan. 01, 2020. doi: 10.1002/ardp.201900272.
23. W. Jamil, S. Solangi, M. Ali, K. M. Khan, M. Taha, and M. Y. Khuhawar, "Syntheses, characterization, in vitro antiglycation and DPPH radical scavenging activities of Isatin salicylhydrazidehydrazone and its Mn [II], Co [II], Ni [II], Cu [II], and Zn [II] metal complexes," Arabian Journal of Chemistry, vol. 12, no. 8, pp. 2262–2269, 2019, doi: https://doi.org/10.1016/j.arabjc.2015.02.015.
24. R. H. Hans et al., "Novel thiolactone–Isatin hybrids as potential antimalarial and antitubercular agents," Bioorg Med Chem Lett, vol. 21, no. 7, pp. 2055–2058, 2011, doi: https://doi.org/10.1016/j.bmcl.2011.02.008.
25. H. M. Osman et al., "Schiff Bases of Isatin and Adamantane-1-Carbohydrazide: Synthesis, Characterization and Anticonvulsant Activity," J Chem, vol. 2021, 2021, doi: 10.1155/2021/6659156.
26. R. Kumar, S. Kumar, and M. Bala, "Synthesis, Characterisation and Antibacterial Activity of Some Indole Derivatives and Their Inclusion Complexes with β-Cyclodextrin," Oriental Journal Of Chemistry, vol. 36, no. 05, pp. 923–933, Oct. 2020, doi: 10.13005/ojc/360518.
27. H. A. Benesi and J. H. Hildebrand, "A Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons," J Am Chem Soc, vol. 71, no. 8, pp. 2703–2707, Aug. 1949, doi: 10.1021/ja01176a030.
28. Job, P. Formation and stability of inorganic complexes in solution. Ann. Chim. 9, 113–203 [1928]
29. Renny, J. S., Tomasevich, L. L., Tallmadge, E. H. & Collum, D. B. Method of continuous variations: applications of Job plots to the study of molecular associations in organometallic chemistry. Angew. Chem. Int. Ed. 52, 11998–12013 [2013].
30. Caso, J. V. et al. Investigating the inclusion properties of aromatic amino acids complexing beta-cyclodextrins in model peptides. Amino Acids 47, 2215–2227 [2015].
31. J. V. Caso et al., "Investigating the inclusion properties of aromatic amino acids complexing beta-cyclodextrins in model peptides," Amino Acids, vol. 47, no. 10, pp. 2215–2227, Oct. 2015, doi: 10.1007/s00726-015-2003-4.

32. M. Cigáň, K. Jakusová, M. Gáplovský, J. Filo, J. Donovalová, and A. Gáplovský, "IPHs: Anion enhanced photochromic behaviour," Photochemical and Photo biological Sciences, vol. 14, no. 11, pp. 2064–2073, 2015, doi: 10.1039/c5pp00275c.
33. L. Tom, C. R. Nirmal, A. Dusthackeer, B. Magizhaveni, and M. R. P. Kurup, "Formulation and evaluation of β-cyclodextrin-mediated inclusion complexes of isoniazid scaffolds: Molecular docking and: In vitro assessment of antitubercular properties," New Journal of Chemistry, vol. 44, no. 11, pp. 4467–4477, Mar. 2020, doi: 10.1039/c9nj06351j.
34. M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," J Pharm Anal, vol. 6, no. 2, pp. 71–79, 2016, doi:https://doi.org/10.1016/j.jpha.2015.11.005.
35. O. Bekircan and H. BeKsas, "Synthesis of Schiff and Mannich bases of Isatin derivatives with 4-amino-4,5-dihydro-1H-1,2,4-triazole-5-ones," Molecules, vol. 13, no. 9, pp. 2126–2135, Sep. 2008, doi: 10.3390/molecules13092126.
36. K. P. Sambasevam, S. Mohamad, N. M. Sarih, and N. A. Ismail, "Synthesis and characterization of the inclusion complex of β-cyclodextrin and azomethine," Int J Mol Sci, vol. 14, no. 2, pp. 3671–3682, Feb. 2013, doi: 10.3390/ijms14023671.
37. A. Roy, S. Saha, B. Datta, and M. N. Roy, "Insertion behavior of imidazolium and pyrrolidinium based ionic liquids into α and β-cyclodextrins: mechanism and factors leading to host–guest inclusion complexes," RSC Adv, vol. 6, no. 102, pp. 100016–100027, 2016, doi: 10.1039/C6RA19684E.
38. K. Sivakumar, T. R. Ragi, D. Prema, and T. Stalin, "Experimental and theoretical investigation on the structural characterization and orientation preferences of 2-hydroxy-1-naphthoic acid/β-cyclodextrin host-guest inclusion complex," J Mol Liq, vol. 218, pp. 538–548, 2016, doi: https://doi.org/10.1016/j.molliq.2016.03.004.
39. P. Mura, "Analytical techniques for characterization of cyclodextrin complexes in the solid state: A review," J Pharm Biomed Anal, vol. 113, pp. 226–238, 2015, doi: https://doi.org/10.1016/j.jpba.2015.01.058.
40. B. Rajbanshi et al., "Study to Probe Subsistence of Host-Guest Inclusion Complexes of α and β-Cyclodextrins with Biologically Potent Drugs for Safety Regulatory Dischargement," Sci Rep, vol. 8, no. 1, Dec. 2018, doi: 10.1038/s41598-018-31373-x.
41. J.-S. Kim, "Study of Flavonoid/ Hydroxypropyl-β-Cyclodextrin Inclusion Complexes by UV-Vis, FT-IR, DSC, and X-Ray Diffraction Analysis," Prev Nutr Food Sci, vol. 25, no. 4, pp. 449–456, 2020, doi: 10.3746/pnf.2020.25.4.449.
42. Upadhyay, S. K., & Kumar, G. [2009]. NMR and molecular modelling studies on the interaction of fluconazole with β-cyclodextrin. Chemistry Central Journal, 3[1]. https://doi.org/10.1186/1752-153X-3-9
43. Y. Liu, Y. Chen, X. Gao, J. Fu, and L. Hu, "Application of cyclodextrin in food industry," Crit Rev Food Sci Nutr, vol. 62, no. 10, pp. 2627–2640, Apr. 2022, doi: 10.1080/10408398.2020.1856035.
44. S. Saha, A. Roy, K. Roy, and M. N. Roy, "Study to explore the mechanism to form inclusion complexes of β-cyclodextrin with vitamin molecules," Sci Rep, vol. 6, Oct. 2016, doi: 10.1038/srep35764.
45. R. Ghosh et al., "Synthesis and characterization of an industrially significant ionic liquid and its inclusion complex with β-cyclodextrin and its soluble derivative for their advanced applications," Chem Phys Lett, vol. 769, p. 138401, 2021, doi: https://doi.org/10.1016/j.cplett.2021.138401.
46. [46] M. N. Roy, S. Saha, S. Barman, and D. Ekka, "Host–guest inclusion complexes of RNA nucleosides inside aqueous cyclodextrins explored by physicochemical and spectroscopic methods," RSC Adv, vol. 6, no. 11, pp. 8881–8891, 2016, doi: 10.1039/C5RA24102B.
47. M. N. Roy, S. Saha, M. Kundu, B. C. Saha, and S. Barman, "Exploration of inclusion complexes of neurotransmitters with β-cyclodextrin by physicochemical techniques," Chem Phys Lett, vol. 655–656, pp. 43–50, 2016, doi: https://doi.org/10.1016/j.cplett.2016.05.031.
48. A. Y. Hao, L. H. Tong, F. S. Zhang, and X. M. Gao, "Convenient preparation of monoacylated β-cyclodextrin [cyclomaltoheptaose] on the secondary hydroxyl side," Carbohydr Res, vol. 277, no. 2, pp. 333–337, 1995, doi: https://doi.org/10.1016/0008-6215[95]00201-4.
49. Y. Izadmanesh and J. B. Ghasemi, "Thermodynamic study of β-cyclodextrin-dye inclusion complexes using gradient flow injection technique and molecular modeling,"

Spectrochim Acta A Mol Biomol Spectrosc, vol. 165, pp. 54–60, 2016, doi: https://doi.org/10.1016/j.saa.2016.04.036.

50. Y. L. Loukas, V. Vraka, and G. Gregoriadis, "Novel non-acidic formulations of haloperidol complexed with β-cyclodextrin derivatives," J Pharm Biomed Anal, vol. 16, no. 2, pp. 263–268, 1997, doi: https://doi.org/10.1016/S0731-7085[97]00029-0.
51. S. B. Carneiro et al., "Cyclodextrin-drug inclusion complexes: In vivo and in vitro approaches," International Journal of Molecular Sciences, vol. 20, no. 3. MDPI AG, Feb. 01, 2019. doi: 10.3390/ijms20030642.