THERMODYNAMIC AND BIOLOGICAL ASSAY OF SCHIFFS BASE ENGULFED β-CYCLODEXTRIN

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Abstract: *Schiff bases/azomethine (-CH=N-) are the privileged moiety as synthesized through facile condensation of carbonys with amines that acted as a guest in inclusion complex formation with the host, Beta-Cyclodextrine (β-Cd). This complexation enhances the solubility of schiffs bases which ultimately augmented innate bioassay profile. In the present study, schiffs base of isatin have been synthesized by condensing with p-anisidine using glacial acetic acid as a catalyst. This biologically potent compound engulfed in the hydrophobic environment of β-Cd via co-precipitation method to yield incusion complex. In solution phase the stoichiometry ratio for this complex was found to be 1:1 using jobs method of continuous variation. The association constant was calculated using modified Benesi-Hildebrand equation for range of temperature. The thermodynamic parameters such as* ΔG^o, ΔH^o *and* ΔS^o *were determined which suggested the feasibility and spontaneity of host-guest association. The relative antimicrobial efficacy of guest in inclusion complex and guest alone were evaluated against three pathogenic bacteria like* Escherichia coli, Staphylococcus aureus, Proteus vulgaris, *and a fungus,* Candida albicans, *using DMSO as a control. The antioxidant assay of schiffs bases and their inclusion compound were studied by DPPH method and found to be elevated on complexation.*

Keywords*: Schiff base, β –Cyclodextrine, Inclusion complex, thermodynamic parameters, antimicrobial studies.*

Introduction:

Supramoleculer chemistry focuses on the host-guest interaction generated through noncovalent connections frequently referred to as "chemistry beyond the molecule." [1] It is an important field, because many biological processes require the host-guest interaction, and it can be useful in some material designs. There are several typical host molecules, such as, cyclodextrin, crown ether, cryptophanes, cryptands, Rotaxane and catenane. Host-guest complexes with CDs have attracted broad interest in the last decades, driven by myriad applications, including drug delivery [2-5] food science [6-7] sensing[8-9] petroleum chemistry [10] and pollutant removal [11-13].

Cyclodextrin (CD), a supramolecular host with 6 (α-CD), 7 (β-CD) and 8 (γ-CD) glucose units connected via (α-1,4) glycosidic linkages, is widely employed in organic chemistry and polymer chemistry research and applications [14]. The chair conformation of repeating glucopyranose units results in CD molecules having a truncated cone shape, with

hydrophobic central cavity and a hydrophilic outer surface. Because of its particular architectural conformation, β-CD can selectively integrate hydrophobic molecules of the suitable size as the guest into its cavity to form water-soluble host–guest inclusion complexes by noncovalent bond [15].

Among the three recognized members, β-Cyclodextrin (β-CD) is the most commonly used host molecule in pharmaceutical formulations due to their favourable cavity size, non-toxicity, biodegradability and economic price. The wide availability and low cost of CDs enables their use in various fields including analysis, catalysis, and surface chemistry, and in many industries such as pharmaceuticals, cosmetics, textiles, and food. A variety of drugs, [16] natural products [17], steroid [18] vitamins[19] oils [20], and other substances have had their physicochemical and biological properties improved by establishing an inclusion complex using B-CD as the host system. The ability of the host B-CD to alter the features of the guest after encapsulation causes advancement in their innate properties.

Isatin, 1H-indole-2,3-dione is a versatile chemical building block, able to form a large number of heterocyclic molecules possesses an extensive range of biological activities [21, 22]. β-Cd have been extensively used as a prominent host system in the inclusion complex formulation with small organic synthesized molecule. In the present study, we discussed the self-assembly inclusion complex formation of Sb, *3-((4-methoxyphenyl)imino)indolin-2-one* into the cavity of β-Cd by coprecipitation method. The inclusion phenomenon was proved by reliable spectroscopy methods whereas the stoichiometry and association constant was determine by Jobs method and Benessi-Hinderband relation respectively .

3. Experimental:

3.1 Material and method:

Perkin Elmer FT-IR spectrometer was employed for taking the Fourier transform infrared (FTIR) spectra of the solutions. The KBr disk of the compound was prepared with 1 mg of the compound and 100 mg of the KBr. A scanning range of 4000–400 cm-1 at room temperature was used to record the FT-IR spectra of the compounds. Bruker AVANCE 400 MHz spectrometer was used to record the 1H NMR spectra of the compounds in DMSO and the signals are mentioned as δ in ppm.

3.2. Synthesis of SB

Isatin and p-anisidine was dissolved in warm ethanol in 1:1 molar ratio. The mixture was heated on a steam bath for 20–40 min. The progress of the reaction was monitor by TLC. After standing for approximately 24 h at room temperature (r.t.), the crystalline products were separated by filtration, vacuum dried and recrystallised from ethanol. [23]

3.3. Synthesis of inclusion complex:

The inclusion complex was prepared in a 1:1 ratio via the co-precipitation method [24] as shown in Fig. 1. 1.135 g (1 mmol) of β-CD was dissolved in 20 mL of de-ionized hot

water. Subsequently, 0.123 g (1 mmol) of SB dissolved in 20 mL hot ethanol was added slowly to the aqueous β-CD solution. The solution was stirred for 2 h at room temperature. The precipitated complex was recovered by filtration and washed with a small amount of ethanol and water to remove unreacted SB and β-CD. The product was dried, collected, and stored in airtight containers for further use.

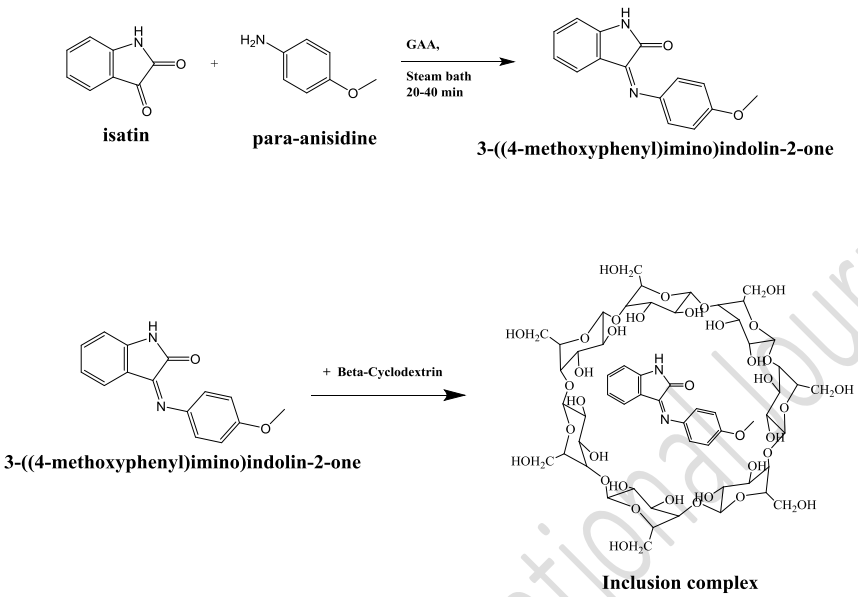


Fig. 1.: Synthesis of schiffs base and its inclusion complex with Beta-Cyclodextrin

4. Result and discussion

4.1. Synthesis of schiffs bases and inclusion complex:

The structural elucidation of synthesised SB and its corresponding inclusion complex was carried out by spectral analysis like Mass, FTIR, and ^{1}H-NMR. The spectral data were in agreement with the assigned structure of SB (Fig. 1c). The melting point of inclusion complexes was found to be higher than that of corresponding guest species, and these preliminary observations directed the formation of inclusion complexes. Physical and analytical data of the schiffs base and inclusion complex is given below:

4.1.1. Schiffs base:

M.F $C_{15}H_{12}N_2O_2$, **Melting point (m.p)** :226 ^{0}C, **% Yeild :**71, **^{1}H NMR (DMSO-d6, δ in ppm) :** 3.7476–3.8710 (s, 3H, -OCH3), 6.7727–7.3327 (m, 8H, 4,5,6,7,2’,3’,5’,6’H), 8.8766 (s, 1H, NH). **FT – IR (υ in cm-1)** : 3948 (O-H),2967 (C-H),2260 (C≡C),1731 (C=O), 1603 (C=C), 1441 (O-H bending),1232 (C-O), 1025 (C-N), 674 (C=C bending), **Mass (m/z)** : Base peak at 253.

4.1.2. Inclusion complex: **Colour**: Orange yellow, **% Yeild**: 64, **Melting point (m.p)** 240^{0}C, **FT – IR (υ in cm^{-1})** 3185 (enolic O-H) 1720 (C=O), 1609 (C=N), 1459,1440 (C=C), 1331 (C-H), 772-708 (Ar-H), **NMR (DMSO-d6, δ in ppm)** (s, 3H, -OCH3)

δ=3.7081–3.7527 (Ar-H, 8H) δ= 6.7717–7.3435 (s, 1H, NH) δ= 8.4024 (β -CD H) δ=3.7081-5.9672

4.2. Stoichiometry:

Job's method of continuous variation has been use to ascertain the stoichiometry of the inclusion complex [25]. Due to the guest molecule's limited solubility in water, all our solutions were created using a DMSO. Solutions were prepared with concentrations ranging from 0.1 mM to 0.01 mM and observed changes in intensity and absorption patterns at maximum wavelength of the guest molecule. To establish the stoichiometry, graph were plotted between the product of difference in absorbance and mole ratio (ΔA×R) against the parameter R, which represents the ratio of the concentration of the guest molecule to the total concentration of guest and host, β-CD.. In this context, ΔA indicates the difference in absorbance between free guest and the guest inclusion complex, while R serves as a measure of the stoichiometry of host-guest inclusion complexes. Specifically, R values of approximately 0.33, 0.5, and 0.66 correspond to host-guest stoichiometries of 1:2, 1:1, and 2:1, respectively.

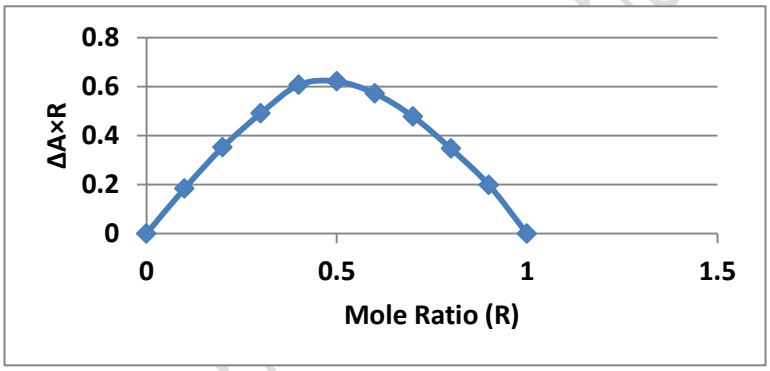


Fig.2 Jobs plot showing 1:1 stoichiometry of host-guest inclusion complex

As the 'Mole ratio (R)' is systematically adjusted from 0 to 1, calculated by dividing the concentration of β-cyclodextrin by the concentration of the guest compound, a clear trend emerges:

The 'A (Absorbance)' values gradually increase (from 0 to 1.925) as the guest concentration rises. The 'ΔA (Change in Absorbance of free guest and guest in the inclusion complex)' demonstrates the incremental changes in absorbance. The 'R × ΔA (Product of Mole Ratio and Change in Absorbance)' values quantify the strength of the interaction, with the highest values observed when the mole ratio is 0.5. The data provides clear evidence of a direct and consistent relationship between the mole ratio (R) and the strength of the interaction between β-cyclodextrin and the guest compound. As the mole ratio approaches 0.5, the interaction becomes notably stronger, signifying the formation of a more robust host-guest complex with 1:1 stoichiometry. These findings offer valuable insights into the dynamics of this specific chemical interaction, which can have significant implications across various scientific and industrial applications.

4.3. Stability constant and allied thermodynamic parameters

The stability constants (Ka) for 1:1 complexation were measured by UV-visible spectroscopy and are presented in Table 1. The association constants of the supramolecular systems formed was calculated according to the modified Benesi-Hildebrand equation Eq. (1) [26-28]

$$\frac{1}{\Delta A} = \frac{1}{\Delta \epsilon} + \frac{1}{Ks[SB]o\Delta\epsilon} \cdot \frac{1}{[\beta-CD]o} \quad (1)$$

Where, [β-CD] and [SB] refer to the total concentration of β-CD and SB respectively, Δϵ is the change in molar extinction coefficient between the free and complexed β-CD and ΔA denotes the absorption changes of SB on the addition of β-CDs. The value of Ka was evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot. The free energy change (ΔG) has been easily estimated using equation 2.

$$\Delta G = -RTlnK \quad (2)$$

The ΔG values (Table. 1) for the complex at all temperature is negative which indicates that the inclusion complex formation is a spontaneous process.

Thermodynamic parameters were calculated basing on the association constant using Vant't Hoff equation 3. [29-30]

$$lnK = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

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

From Vant Hoff's equation 3, the plot between 1/lnk and 1/T shows a linear relationship (Fig.2) The results are summarised in Table 1.

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

T (K)	1/T (K)	Ka	Lnks	Slope	intercept	ΔG° (KJ/mol)	ΔH° KJ/mol)	ΔS° (KJ/mol)
298.15	0.003354	1544.85	7.342682	5759.4	-11.941	-18.2	-47.883	-0.099
303.15	0.003299	1206.87	7.095786					
308.15	0.003245	877.8	6.777419					
313.15	0.003193	613.58	6.419311					

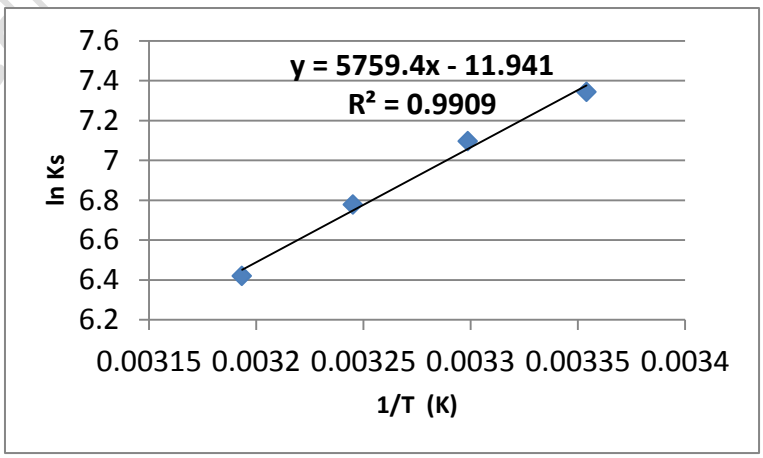


Fig. 3: Plot between 1/lnk and 1/T shows a linear relationship.

4.4. Antimicrobial studies:

The antimicrobial activity of synthesized Schiff base and corrosponding inclusion complex with β-cyclodextrin was tested using Agar well diffusion method for microorganisms such as S. aureus, Pseudomonas auregenous, S. typhi, and Candida albicans. [31]. The antimicrobial profile of the inclusion complex against the pathogens under study was found to be significantly stronger than that of the guest alone. Among the microorganisms under examination, the guest SB and its inclusion complex, β-Cd/SB, demonstrated a distinct efficacy against S. aureus, exhibiting 10 mm and 11 mm zones of inhibition at 100 ug/ml, respectively. The antifungal effectiveness of the guest against Candida albicans increases when the SB forms a complex with β-CD. 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 improved antimicrobial qualities . The inclusion product's decreased polarizability increased its lipophilicity relative to SB, which decreased the permeability of the cells and interfered with normal cell functions. Figures 4a–d represents the graphical summary of the findings.

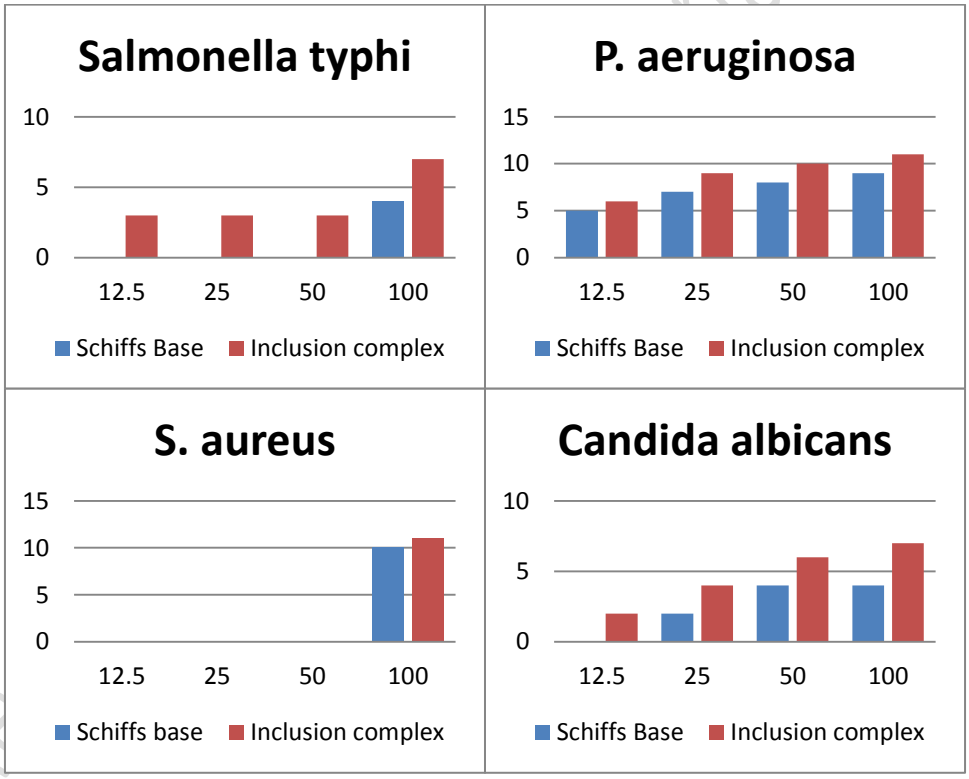
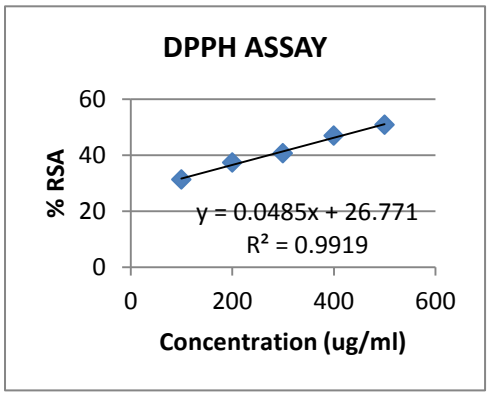


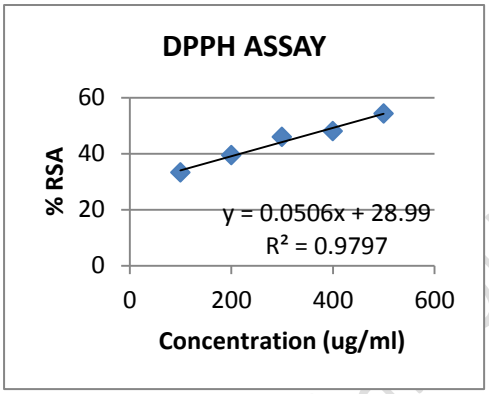
Fig.4. Graphical summary of antimicrobial activity of schiffs base and inclusion complex against Salmonella typhie, P. aeruginosa, S. aureus and Candida albicans

4.5. Antioxidant studies:

The guest schiffs base and its inclusion compound were screened for antioxidant activity by the DPPH assay method using ascorbic acid as standard [32]. The DPPH assay method depends on the reduction of purple DPPH to a yellow colored diphenylpicrylhydrazine and the remaining DPPH which showed maximum absorption at 517 nm is measured.The results

of free radical scavenging activity of compounds in different concentrations are shown in Table. 02 . The results predict the free radical scavenging activities of guest schiffs base and its inclusion complex are concentration dependent and found to be superior after encapsulation. The guest and its inclusion complex showed a strong interactive ability with DPPH which is concentration dependent.





(4a) **(4b)**

Fig. 4. Antioxidant activity of (4a) schiffs base and its (4b) inlcuison complex in term of % RSA and their IC50 values.

Concentration (ug/ml)	Radical scavenging activity (% RSA)	
	Schiffs base	Inculsion complex
100	30.12	35.68
200	37.47	44.98
300	44.18	53.67
400	57.08	61.52
500	63.7	67.25
IC50	340.1	267.051

5. Conclusion:

This paper describes the formation of an inclusion complex with β-CD in an aqueous media using the pharmacologically active isatin Schiff base. Job's graphs demonstrate their 1:1 stoichiometry, and spectroscopic experiments demonstrated the synthesis of the guest schiff base and its inclusion complex. It is shown that the formation of inclusion complexes is an exothermic, spontaneous process by measuring the association constants and thermodynamic parameters with a UV-Vis spectrophotometer. Its antibacterial and antioxidant properties were further boosted by the inclusion complex formation of the guest with β-CD. Hence, the current study offers a qualitative and quantitative comprehension of how the inclusion complex of β-CD forms with isatin Schiff's base, suggesting potential applications in the pharmacological and medical domains.

Reference:

- Huang, Feihe, and Eric V. Anslyn. 2015, "Introduction: supramolecular chemistry, Chemical reviews, 115.15:6999-7000.
- S. V. Kurkov and T. Loftsson, 2013, Int. J. Pharm., 453, 167–180
- Z. Ren, Y. Xu, Z. Lu, Z. Wang, C. Chen, Y. Guo, X. Shi, F. Li, J. Yang and Y. Zheng, 2019, RSC Adv., 9, 11396–11405
- Ogawa N, Takahashi C, Yamamoto H. (2015). Physicochemical characterization of cyclodextrin-drug interactions in the solid state and the effect of water on these interactions. Journal of Pharmaceutical Sciences, 104, pp. 942–954
- Liu BW, Zhou H, Zhou ST, Yuan JY. (2015). Macromolecules based on recognition between cyclodextrin and guest molecules: synthesis, properties and functions. European Polymer Journal, 65, pp. 63–81.
- Astray G., Mejuto J.C., Simal-Gandara J. 2020; Latest Developments in the Application of Cyclodextrin Host-Guest Complexes in Beverage Technology Processes. Food Hydrocoll. 106:105882. doi: 10.1016/j.foodhyd.2020.105882.
- Matencio A., Navarro-Orcajada S., García-Carmona F., López-Nicolás J.M. 2020; Applications of Cyclodextrins in Food Science. A Review. Trends Food Sci. Technol. 104:132–143. doi: 10.1016/j.tifs.2020.08.009.
- Ogoshi T., Harada A. 2008 ;Chemical Sensors Based on Cyclodextrin Derivatives Sensors. 8:4961–4982. doi: 10.3390/s8084961.
- Zhu G., Yi Y., Chen J. 2016; Recent Advances for Cyclodextrin-Based Materials in Electrochemical Sensing. Trends Anal. Chem. 80:232–241. doi: 10.1016/j.trac.2016.03.022.
- Tang W., Zou C., Da C., Cao Y., Peng H. 2020,A Review on the Recent Development of Cyclodextrin-Based Materials Used in Oilfield Applications. Carbohydr. Polym. ;240:116321. doi: 10.1016/j.carbpol.2020.116321.
- Liu Q., Zhou Y., Lu J., Zhou Y. 2020; Novel Cyclodextrin-Based Adsorbents for Removing Pollutants from Wastewater: A Critical Review. Chemosphere241:125043. doi: 10.1016/j.chemosphere.2019.125043.
- Tian B., Hua S., Tian Y., Liu J. 2021; Cyclodextrin-Based Adsorbents for the Removal of Pollutants from Wastewater: A Review. Environ. Sci. Pollut. Res. 28:1317–1340. doi: 10.1007/s11356-020-11168-2.
- Geue, N.; Alcázar, J.J.; Campodónico, P.R. (**2023)** Influence of β-Cyclodextrin Methylation on Host-Guest Complex Stability: A Theoretical Study of Intra- and Intermolecular Interactions as Well as Host Dimer Formation. *Molecules* , *28*, 2625. https://doi.org/10.3390/molecules28062625
- C. Zou, P. Zhao, J. Ge, Y. Lei, P. Luo, (2012), β-Cyclodextrin modified anionic and cationic acrylamide polymers for enhancing oil recovery, Carbohydr. Polym. 87 607-613.
- Carneiro, S.B.; Costa Duarte, F.Í.; Heimfarth, L.; Siqueira Quintans, J.d.S.; Quintans-Júnior, L.J.; Veiga Júnior, V.F.d.; Neves de Lima, Á.A. (2019), Cyclodextrin–Drug Inclusion Complexes: In Vivo and In Vitro Approaches. *Int. J. Mol. Sci. 20*, 642. https://doi.org/10.3390/ijms20030642

- Butnariu, M., Peana, M., Sarac, I. et al. (2021). Analytical and in silico study of the inclusion complexes between tropane alkaloids atropine and scopolamine with cyclodextrins. Chem. Pap. **75**, 5523–5533.https://doi.org/10.1007/s11696-021-01742-4
- Nina Sadlej-Sosnowska, Molecular complexation: β-cyclodextrin and steroid hormones inclusion complexes studied by high performance liquid chromatography, European Journal of Pharmaceutical Sciences, Volume 3, Issue 1, Pages 1-5, https://doi.org/10.1016/0928-0987(94)00006-L.
- Saha, Subhadeep, et al. (2016) "Study to explore the mechanism to form inclusion complexes of β-cyclodextrin with vitamin molecules." Scientific reports 6.1: 35764.
- Farouk, A.; Sharaf, S.; Refaie, R.; Abd El-Hady, (2022). M.M. Highly Durable Antibacterial Properties of Cellulosic Fabric via β-Cyclodextrin/Essential Oils Inclusion Complex. Polymers , 14, 4899. https://doi.org/10.3390/polym14224899
- Bhrigu B, Pathak D, Siddiqui N, Alam MS, Ahsan W, (2010) Search for biological active isatins: a short review. Int J Pharm Sci Drug Res, 2, 229–235.
- Malhotra S, Balwani S, Dhawan A, Singh BK, Kumar S, Thimmulappa R, Biswal S et al.: (2011) Synthesis and biological activity evaluation of N-protected isatin derivatives as inhibitors of ICAM-1 expression on human endothelial cells. Med Chem Commun, 2, 743–751.
- L. Tom, C. R. Nirmal, A. D. V. N., M. (2020), Balasubramanian and M. R. P. Kurup, New J. Chem., DOI: 10.1039/C9NJ06351J.
- Cramer, F., Saenger, W., & Spatz, H. C. (1967). Inclusion compounds. XIX. 1a The formation of inclusion compounds of α-cyclodextrin in aqueous solutions. Thermodynamics and kinetics. Journal of the American Chemical Society, 89(1), 14-20.
- L. Yuan, S. Li, D. Huo, W. Zhou, X. Wang, D. Bai, J. Hu, (2019), Studies on the preparation and photostability of avobenzone and (2-hydroxy)propyl-β-cyclodextrin inclusion complex, Journal of Photochemistry and Photobiology A: Chemistry, 369, 174-180.
- D. Maya, C. Olea-Azar, (2019), Supramolecular hydrogels of β-cyclodextrin linked to calcium homopoly-l-guluronate for release of coumarins with trypanocidal activity, Carbohydrate Polymers, 204,170-181.
- S. Saha, A. Roy, M.N. Roy, (2017) Mechanistic Investigation of Inclusion Complexes of a Sulfa Drug with αand β-Cyclodextrins, Industrial & Engineering Chemistry Research, 56 11672-11683.
- S. Saha, A. Roy, K. Roy, M.N. Roy, (2016), Study to explore the mechanism to form inclusion complexes of βcyclodextrin with vitamin molecules, Scientific Reports, 6 35764.
- R.K. Dubey, U.K. Dubey and S.K. Mishra, J. Coord. Chem., 2011, 64, 2292-2306
- Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. (2022) Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules.;27(4):1326. doi: 10.3390/molecules27041326.