CHAPTER VIII

INCLUSION OF HYDRAZINOPHTHALAZINE INSIGHT INTO THE CAVITY OF β-CYCLODEXTRIN: A STUDY OF SURFACE TENSION AND UV-VIS SPECTROSCOPY

Abstract

Surface tension, conductivity, and ultraviolet spectroscopic methods have been employed to study the dimensional fit of molecular encapsulation of hydrazinophthalazine hydrochloride insight into the cavity of β-cyclodextrin in aqueous media. The equilibrium constant and 1:1 stoichiometry of the complex has been analyzed by surface tension (plot against reciprocal of concentration), and Job's plot (drawn from UV-Vis data). The binding constants computed from the tensiometric and spectroscopic method were found to be 59.91μM^{-1} and 12.02-96.58μM^{-1}, respectively, that return the comfort zone of the results. The noteworthy upshot has also come out from standard Gibbs energy for inclusion complex formation. The free energy for inclusion complex formation is negative and lower in magnitude than adsorption by 6.11kJ mol^{-1}. ^{1}HNMR and SEM picture also certified the formation of the inclusion complex. The results demonstrated that the driving force for formation of the inclusion complex inside the bucket-like cavity of β-cyclodextrin was a combination of hydrophobic effect and reduction of the surface energy, while in adsorption is only hydrophilic effect.

Keywords:

Hydrazinophthalazine hydrochloride; β-cyclodextrin; surface tension; UV-vis spectroscopy; inclusion complex

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1. Introduction

Inclusion complex formation is a dimensional fit of host molecule insight into the host cavity, the phenomena can also be termed as molecular recognition. [1,2] Cyclodextrin and its derivative's inclusion phenomena achieved broad range of application in *in-vivo* and *in-vitro.* [3-6] The inner cavity cyclodextrin is principally hydrophobic while the outer phase is hydrophilic having a good response in aqueous solubility. The polarity difference between the interior and exterior of the cavity provide it encapsulating power. On the other side, poorly bounded two molecules of water present in the cavity [7] of β-cyclodextrin (βCD), provide it excellent driving force to encapsulate the hydrophobic moiety of the guest molecule. This property makes it to be one of the outstanding drug carrier. [8,9] It is also used as solubility enhancer[10-12], stabilizing agent[13,14], hydrophobic group protector[15], toxicity reducing agent [16,17], catalysts for green synthesis [18,19] etc.

Hydrazinophthalazine hydrochloride (also known as hydralazine) [20] molecule, belongs to the cyclic hydrazine family. It is one of the potential therapeutic drug for hypertension[21], which composed of 10, 25, 50, or 100 mg tablets for oral dose. [22] The main function of the drug is, in midbrain it clam the pituitary hormone that acts to promote the retention of water by the kidneys and increase the blood pressure. Its hydrochloride salt is effective drug, whose condign dose reduces the arterial blood pressure and peripheral vascular resistance, whereas increases the rate of heart beats and the amount of blood pumped out by the ventricles in a given time period. [23] Therefore it is often prescribed to minimize abnormally high blood pressure in large number of patients. Albeit, it used as tumorigenic effect in mice [23], to control on hypertensive preeclampsia [24], as waste water treatment [25], for synthesis of chemosensor NNI [26] etc.

A fruitful understanding of the mechanism and basic principles that govern on the host-guest complexation between hydrazinophthalazine hydrochloride and β-cyclodextrin helps us to customise the composition, for designing a better drug delivery system coupled with increased therapeutic potential. After the thorough survey, it has been seen that there are no articles published on this topic. Therefore, to make the clear knowledge about the mechanism of encapsulation between hydrazinophthalazine and

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β-cyclodextrin, in the present investigation we have employed surface tension and UV-vis spectroscopic data. Here, we have deduced the stiochiometry of the complex, binding constant, standard free energy change and binding nature and discussed them with reference to molecular recognition.

Aim of this work is the formation, carrying and controlled release of hydrazinophthalazine (HP) by inclusion complex with host cyclodextrin molecules without chemical and biological distortion.

2. Experimental section

2.1 Materials

1-hydrazinophthalazine hydrochloride and β-cyclodextrin of puris grade were purchased from Sigma-Aldrich and have been used without further purification. Triple distilled water having the conductance $1 \cdot 10^{-6}$ S·m^{-1} has been used for the preparation of the solution.

2.2 Apparatus

Surface tension of the solutions has been measured using platinum ring detachment technique with a K9 digital tensiometer (Krüss GmbH, Hamburg, Germany) at the experimental temperature. The accuracy of measurement is ±0.1mN·m^{-1}. Temperature has been maintained by the circulation of auto thermostat water through a double-walled glass vessel containing the solution.

UV–visible spectra were recorded using a JASCO V-530 UV–VIS spectrophotometer having a wavelength accuracy of ±0.5nm. A digital thermostat was used to maintain the cell constant and temperature.

2.3 Procedure

Before executing the practical experiment, the solubility of host and guest molecules have been checked. 1-Hydrazinophthalazine hydrochloride and β-cyclodextrin, both are soluble in water (triple distilled). The solutions were prepared by mass measurements

(accomplished by using a Mettler AG-285 electronic balance with a precision of ±0.0003x10^{-3} kg) in aqueous solution. Precautions were taken to minimize weight loss and the uncertainty in molality of solution was found to be ±0.0001 mol kg^{-1}. All the solutions were prepared freshly before performing experimental measurement.

2.4 Apparatus

Surface tension of the solutions was measured using the platinum ring detachment technique with a K9 digital tensiometer (Krüss GmbH, Hamburg, Germany) at the experimental temperature. The accuracy of measurement was ±0.1mN·m^{-1}. Temperature was controlled using a circulation of auto thermostat water through a double-walled glass vessel containing the solution. UV–visible spectra were recorded using a JASCO V-530 UV–VIS spectrophotometer having a wavelength accuracy of ±0.5 nm. A digital thermostat was used to maintain the cell constant and temperature. METTLER TOLEDO-7 multi conductivity meter has used for the measurement of specific conductivity values with an uncertainty of ±1.0 μS m−1 . 1H NMR spectra has been recorded in D2O using Bruker Avance 400 MHz instrument. Signals have been cited as δ values in ppm with reference to residual protonated solvent signal as the internal standard (HDO, δ 4.79 ppm). Data have presented in chemical shifts. Using JEOL JSM-IT 100 Scanning Electron Microscope (SEM), the surface morphologies of host, guest and complexes have recorded. The pictures were taken at an excitation voltage of 30kV with a magnification of 2000X.

3. Results and Discussion

3.1. Surface tension method

3.1.1. Inclusion, adsorption and thermodynamic parameters

The experiments were carried out at pH 3.4 - 4.9 to ensure the predominant presence of the guest and involved in complexation with β-CD. Here, the assumption has taken as only hydrazinophthalazine (HP) is contributing for inclusion, and the hydrochloride or chloride are stabilising the complex or act as a counter ions. Incoming hydrazinophthalazine molecules in aqueous solution of β-CD experience two scenarios,

(a) adsorption at air-water interfaces and (b) inclusion complex formation or other associative behavior (diagram VIII.1). The rest non-associative phase has been conjectured as bulk solution. Both the scenarios, viz., surface adsorption and inclusion are manifested by surface tension, association or binding constant and free energy change. With respect to concentration, the surface tension (γ) data have been represented in Figure VIII.1.

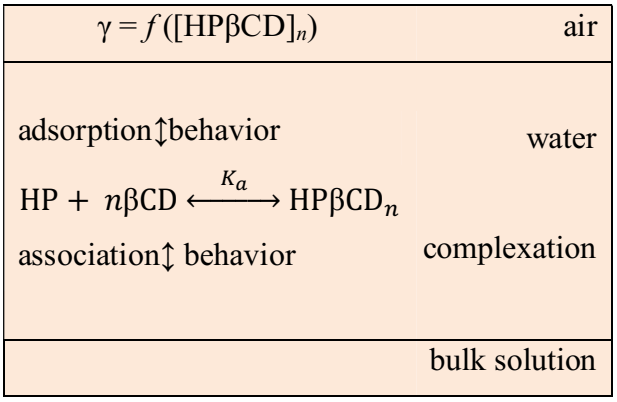

Diagram VIII.1. Scenarios of interaction behaviour of hydrazinophthalazine and β-CD

The figure showing two types of nature of γ, where both the curves intersecting at a same point, that kink has been considered as saturation point of inclusion complex (γ_{ic}). The corresponding concentration is called as saturation point of inclusion complex concentration (C_{ic}). Here, the γ_{ic} and C_{ic} are 79.4mN·m^2 and 5.01mM respectively (table VIII.1). Below the C_{ic}, the incorporation of hydrazinophthalazines are occurring insight into the apolar cavity of β-CD, in addition to adsorption. We have considered the adsorption here to be a physical adsorption rather than a chemical adsorption. At this juncture, the inclusion is surface active; the number of surface adsorption at the air/water interface is enhanced upon injection of β-CD (scheme VIII.1). Above that the variation is almost parallel to *x*-axis, this is because there is no guest molecule present for inclusion, therefore, the graph is only for aqueous β-CD, and we know that cyclodextrin is not surface active compound, in aqueous solution it does not significantly change the surface tension. [27]

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Surface tension data has been plotted with logarithm of concentration and Gibbs isotherm equation used to determine the surface excess (Γ),

$$|\Gamma| = \frac{1}{RT}\frac{d\gamma}{dlnC} \quad (1)$$

Equation 1 can also be rearranged as,

$$d\gamma = RT\,|\Gamma|\,dlnC \quad (2)$$

On integration, equation 2 shows the surface tension is a function of concentration. We have assumed that the change of adsorption enthalpy unaltered. Therefore, the Langmuir isotherm [28] can be written as,

$$\theta|\Gamma| = \frac{K_{ad}C}{1+K_{ad}C} \quad (3)$$

where, θ and K_{ad} are the cross-section area of the hydrazinophthalazine molecules at the surface and equilibrium constant for adsorption respectively. Evaluate the surface excess from equation 3, substituting into Equation 2 and after integration gives the Szyszkowski equation [29] in positive variant.

$$\gamma = \gamma_o + \frac{RT}{\theta}\ln(1 + K_{ad}C) \quad (4)$$

Here, the surface tension of pure water is γ_o (70.8 mN·m^{-1}). Fitting the data of known parameters to equation 4, the cross-section area occupied by hydrazinophthalazine molecules at the surface and surface adsorption equilibrium constant below the saturation of complexation has been determined. For simplifying the calculation of equation 4, we have predefined the value of K_{ad}, so that the intercept can be fitted to 70.8 mN·m^{-1}. Then, θ has been evaluated from the slope. From Table VIII.1, the considerable cross-section area occupied by the molecules with a strong adsorption constant at the surface so as to form the complex. The standard Gibbs energies for adsorption and inclusion complex formation have been computed using surface

adsorption equilibrium constant and inclusion complex concentration (C_{ic}) respectively [30],

$$\Delta G^{o}_{ad} = -R \quad ln\, K_{ad} \;\; and\; \Delta G^{o}_{ic} = -R \quad ln\, C_{ic} \tag{5}$$

The standard Gibbs energy for inclusion complex formation is more negative than for adsorption by 6.11 kJ mol^{-1}. That make sense, the driving force in adsorption is only hydrophilic effect, while in formation of inclusion complex it is a combination of hydrophobic effect and reduction of the surface energy.

3.1.2. Guest : Host binding stoichiometry and binding constant

The argument on the binding stoichiometry between hydrazinophthalazine and β-CD has been taken into account in discussion. The major conflict is, whether the binding ratio is 1:1 or 2:1. The ratio has been optimized by employing the surface tension data on modified Benesi-Hildebrand equation. The equation has modified on the complex formation between hydrazinophthalazine and βCD as follows,

$$HP + n\;\beta CD \xrightarrow{K_a} \mathrm{HP}\beta CD_n$$

The binding constant K_a is given by,

$$K_a = \frac{[\mathrm{HP}\beta CD_n]}{[HP][\beta C\;\;]^n} \tag{6}$$

where, n is the number of cyclodextrin participating to complex formation with respect to one molecule of hydrazinophthalazine. If the analytical concentration of the host and guest are ($[HP]_o$) and ($[\beta CD]_o$) respectively,

$$[HP]_o = [HP] + [\mathrm{HP}\beta CD_n]$$

$$\text{and} \quad [\beta CD]_o = [\beta CD] + [\mathrm{HP}\beta CD_n]$$

then K_a can be rewritten as,

$$K_a = \frac{[\mathrm{HP}\beta CD_n]}{\{[HP]_o - [\mathrm{HP}\beta CD_n]\}\{[\beta CD]_o - [\mathrm{HP}\beta CD_n]\}^n} \quad (7)$$

In experiment, we have used large excess of βCD (100mM) relative to constant concentration of hydrazinophthalazine (10mM). Thus, we may assume $[\beta CD]_o \gg [\mathrm{HP}\beta CD_n$ or $\{[\beta CD]_o - [\mathrm{HP}\beta CD_n\} \approx [\beta CD]_o$. Then, equation 7 reduces to,

$$K_a = \frac{[\mathrm{HP}\beta CD_n]}{\{[HP]_o - [\mathrm{HP}\beta CD_n]\}[\beta CD]_o{}^n} \quad (8)$$

At a low concentration of hydrazinophthalazine, it is observed that the surface tension is proportional to complex concentration.

$$\gamma_{[\mathrm{HP}\beta CD_n]} = r\,[\mathrm{HP}\beta CD_n] \quad (9)$$

Where, r is the proportionality constant. Substituting the $[\mathrm{HP}\beta CD_n]$ from Equation 9 into Equation 8, and rearranging we have

$$\frac{[HP]_o}{\gamma_{[\mathrm{HP}\beta CD_n]}} = \frac{1}{r\,K_a}\frac{1}{[\beta CD]_o{}^n} + \frac{1}{r} \quad (10)$$

Substituting $\gamma_{[\mathrm{HP}\beta CD_n]} = \gamma - \gamma_o$ (here, γ_o and γ are surface tension of the solution in absence and presence of βCD), equation 10 reduced to

$$\frac{[HP]_o}{\gamma - \gamma_o} = \frac{1}{r\,K_a}\frac{1}{[\beta CD]_o{}^n} + \frac{1}{r} \quad (11)$$

Figure VIII.2 depicts the Plot of $\frac{[HP]_o}{\gamma - \gamma_o}$ against $\frac{1}{[\beta CD]_o{}^n}$ for n=1, 2, 3 etc, and the nature of the curve found out. Linear plot play the best fit for the whole number binding stoichiometry (n), while the nonlinear plot diverge the correct stoichiometry. Perusal of Figure VIII.2 disclose that the modified Benesi-Hildebrand plot is linearly fit only for n=1, and curve for n=2 and higher. So, it is confirmed that only 1:1 stoichiometric

complexation are occurring; in other word, at a time only one hydrazinophthalazine molecule can be integrated insight into the cavity of βCD. The binding constant (K_a) has been computed from the ratio of intercept to the slope; and free energy change (ΔG_a^o) from K_a. From Table VIII.1, the K_a is 14 times higher than K_{ad}; in contrast to ΔG_a^o is 20% and 45% lesser than ΔG_{ic}^o and ΔG_{ad}^o respectively. These findings reveal that the inclusion complex is formed by strong binding between incorporated hydrazinophthalazine and β-CD. The complexation is stabilized in lower energy even than adsorption. Comparing the considerable these three types of standard free energy (ΔG_{ic}^o, ΔG_{ad}^o, and ΔG_a^o,), it can be said that both the adsorption and incorporation are occurring in a same extent from end to end via opposite direction.

3.2. UV-Vis spectroscopic method

The UV-Vis spectral data plays an incomparable method for analysis of inclusion behavior of host-guest complex. Here, UV-vis spectral data have been employed to evaluate the stoichiometry of the complexation and binding association. Stoichiometry had manifested by dint of Job's method which is also known as continuous variation method, while binding association was determined with the help of Benesi-Hildebrand equation.

3.2.1. Job plot for determination of stoichiometry of guest : host inclusion complex

A set of stock solutions (hydrazinophthalazine + β-CD) have been formulated separately By varying the molar ratio viz., 4ml:0ml, 3.6ml:0.4ml, 3.2ml:0.8ml and so on with keeping the total concentration of species constant. The absorption spectra at λ_{max} have been observed for each set of solution at room temperature (T=298.15K). Then, Job's plot generated by plotting ($R{\cdot}\Delta A$) against R, where ΔA is the absorption difference of hydrazinophthalazine in presence and absence of βCD, and $R = [HP]/\{[HP]+[\beta CD]\}$. R provides the stiochiometry of the complexes. As for example, when R = 0.25, the ratio of the guest and host complex is 1:3; similarly, the mole fraction 0.33, 0.50, 0.66, and 0.75, assigned 1:2, 1:1, 2:1, and 3:1 respectively. In Figure VIII.3, we have shown a plot of $R{\cdot}\Delta A$ *vs* R for corresponding absorption maxima at 271 nm. A half oval-shaped has been

observed from in Figure VIII.3, where $R \cdot \Delta A$ is maximum at R = 0.50. This implies, 1:1 stoichiometric guest-host inclusion complex has formed.

3.2.2. Binding constant and free energy change

The degree of encapsulation and retention of the guest molecule in the interior cavity of β-CD can be explained by stability of the complex, binding nature, binding strength and standard free energy change. Binding potency in term of constant of inclusion (K_a) has been explored by Benesi-Hildebrand method for 1:1 complex. The double reciprocal plot has been obtained following the equation,

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon\,[HP]K_a}\,\frac{1}{[\beta CD]} + \frac{1}{\Delta\varepsilon\,[HP]} \qquad (12)$$

where, ΔA attributes to the difference of guest absorbance before and after complexation with β-CD. In order to compute the binding constant, changes in absorbance (ΔA) were plotted against wave length (λ) at different concentration (0 to 2.8 mM) of β-CD (Figure VIII.4). The absorbance maxima have been observed at 208 nm, 245 nm and 299 nm. If we notice the λ_{max} of hydrazinophthalazine is at 211, 240, 260, 304, and 315 nm (where, β-CD is UV inactive), the absorbance maxima of complex at 208 nm shows blue shift with respect to 211 nm; and 245 nm and 299nm are red shift with respect to 240 nm and 260 nm respectively. This is due to π - π* electron transition between the aromatic ring. Double reciprocal plot of $\frac{1}{\Delta A}$ vs $\frac{1}{[\beta CD]}$ has been plotted at 208 nm, 245 nm and 299 nm and represented at Figure 5. Binding constant (K_a) has been calculated by dividing slope by intercept at 208 nm. Similar way has employed at 245 nm and 299 nm and the magnitudes have listed in Table VIII.1. After that standard free energy changes (ΔG_λ^o) have been worked out by means of K_a. Scrutiny of the Table VIII.1, expose very high values of K_a than estimated from surface tension; on the other hand these are extremely high than adsorption constant. The results are in line with surface measurement that association/binding capacity of inclusion is higher than adsorption. Alternatively, the standard free energy changes (ΔG_λ^o) are more or less lie within the range, as obtained from surface tension computation. Overall, it can be attributed that 1:1 ratio is powerful complexation with great stability and feasible at lower energy.

*Published in Asian Journal of Green Chemistry 5 (2021) 183-195

3.3. Conductivity data scrutiny

Conductivity data is also an important tool to elucidate the inclusion phenomenon in the solution. [32] From the scrutiny of the data, it is obvious that specific conductance (κ) is decreasing gradually, due to the fact of encapsulation of charged HPHC molecules into the β-CD cavity. Single break in the conductivity curve, at 5 mM of β-CD, suggests that hydrazinophthalazine and βCD complex with equimolar ratio; and therefore, the host-guest stoichiometry for the complaxation is 1:1.

3.4. Structural feature of guest and host molecules

Complex formation between the guest's hydrophobicity and host's apolar cavity can only make the clear sense if we demonstrate the fitting with respect to their molecular dimension or volume. The noticeable feature of the inner cavity size is 6.0Å – 7.0Å [31] (6.0-6.5Å [7]) of the β-cyclodextrin molecule provides a potential environment, in which appropriately sized apolar moiety of the guest meet and form stable complex. The qualified dimension of hydrazinophthalazine [33] is 5.25Å (apolar benzene ring) and 6.6 Å (two unit of benzene ring+side chain of hydrazine part) (Scheme 2), where the apolar moiety viz., benzene rings are eligible for incorporation in the cavity. According to the dimensional fitting approach or in view of the dimension of both the apolar part of guest and space of cavity, it is confirmed that only one hydrazinophthalazine moity can occupy the cavity of one cyclodextrin molecule. In other word we may say that there is only 1:1 stoichiometric complaxation are occurring. The tendency of the occupation is driven by the water molecules present into the cavity of cyclodextrin. [34] Because, in cavity, the water molecules are highly energetic and unfavourable, consequently they are substituted and pull the hydrophobic moiety of guest (which is less polar). Where, they undergo hydrophobic-hydrophobic interactions and cyclodextrin ring strain is diminished, as a result the complex becomes stable with lower energy state. But, it should be noted that during the complex formation there are no covalent bonds breaking or forming with retention of configuration of cyclodextrin. They are attached via non-covalent bonds like hydrogen bond, van der Waal force, electrostatic force, hydrophobic interactions etc. While, the –NH=NH_2 is arrested at the periphery by secondary –OH groups of cyclodextrin through H-bond or electrostatic force or hydrophilic-hydrophilic interactions. Hence, the plausible formation of 1:1 guest-host

ratio as if by magic molecular encapsulation is remarkably in agreement with the experimental data obtained from surface tension and UV-Vis spectroscopy.

3.5 ^{1}H NMR analysis of complex

The protons in βCD are in different environments. From the side of cavity, H3' and H5' proton is located near the wider and narrower rim respectively; whereas H1', H2' and H4' are oriented at the exterior of CD molecule (Scheme VIII.3). 1HNMR spectra of complex, β-CD, and pure hydrazinophthalazine are represented in Figure VIII.7. Where, the signals of the interior H3' and H5' protons of CD as well as the interacting aromatic protons of hydrazinophthalazine showed down-field shifts (Table VIII.2 and Figure VIII.7), that confirms the formation of complex. Higher value of the chemical shift (δ) in case of H3' proton than H5', also provide the information that the guest choose the wider rim to incorporate into the cavity of β-CD (Scheme 2).

3.6. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) used to analyze the surface texture and particle size of the complex. The surface morphology of pure hydrazinophthalazine, pure β-CD, and inclusion complex are shown in Figure VIII.8. A vast difference has seen between the morphological structures of pure hydrazinophthalazine or β-CD and the complex. So, this is one of the evidence that indicates the strong complexation again.

4. Conclusions

From the observed results and discussion it can be concluded that hydrazinophthalazine molecule is incorporated insight into the truncated cone type hydrophobic cavity of β-cyclodextrin molecule to form 1:1 stoichiometric complex. Evaluation of binding stoichiometry and constants from both the surface tension and UV-vis spectroscopy demonstrated the same observation that the host-guest inclusion complex formed with great stability. The complex has been formed by replacing the water molecules present into the cavity of cyclodextrin (which make a driving force for incorporation of guest molecule) and becomes stable with non-covalent interaction. The inclusion complex formed balance both surface and bulk properties of solution by

adsorption and inclusion phenomena. But the inclusion phenomena are dominant over the surface adsorption.

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TABLES

■ **Table VIII.1.** Thermodynamic parameters computed from surface tension and uv spectroscopy

Parameters	Numerical values	Parameters	Numerical values
C_{ic}	: 5.01 mmolL^{-1}	Average λ_{max}	: λ_1 = 208nm
ΔG^o_{ic}	: -19.24 kJ mol^{-1}		: λ_2 = 245nm
K_{ad}	: 4.26×10^{-4} molL^{-1}		: λ_3 = 299nm
θ	: 3.53×10^{-5} m^{2}mol^{-1}	$^{\#}K_a$ at 208nm	: 12.03×10^3 molL^{-1}
ΔG^o_{ad}	: -13.13 kJ mol^{-1}	$^{\#}K_a$ at 245nm	: 96.58×10^3 molL^{-1}
$^{*}K_a$	: 59.91 ×10^{-3} molL^{-1}	$^{\#}K_a$ at 299nm	: 22.84×10^3 molL^{-1}
ΔG^o_a	: -24.10 kJ mol^{-1}	$\Delta G^o_{\lambda 1}$	: -23.29 kJ mol^{-1}
		$\Delta G^o_{\lambda 2}$	: -28.45 kJ mol^{-1}
		$\Delta G^o_{\lambda 3}$	: -24.88 kJ mol^{-1}

*surface tension and using C^{-n} method; $^{\#}$uv spectroscopy method

■ **Table VIII.2.** Chemical shifts (**δ**/ppm) of protons of β-Cyclodextrin after complex formation

Protons	**δ** βCD	**δ** (βCD+HP)	**Δ δ** (βCD+HP)
$H^{3'}$	3.867	3.832	0.35
$H^{5'}$	3.779	3.758	0.21

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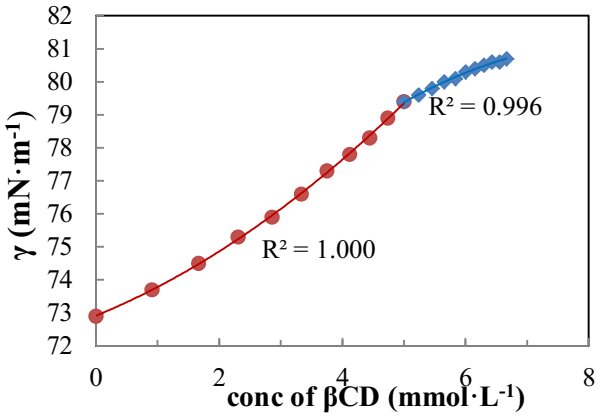
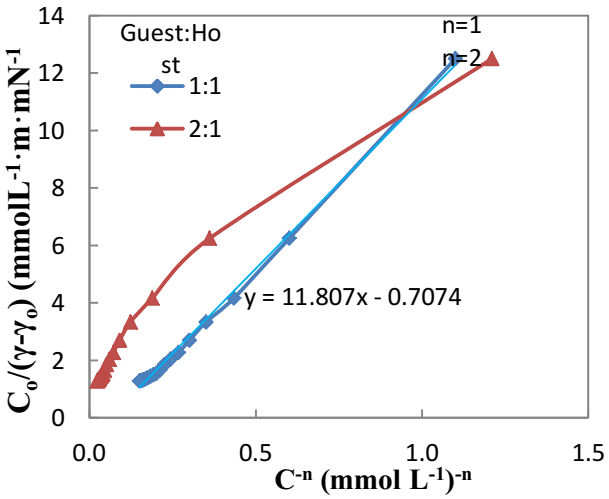
FIGURES

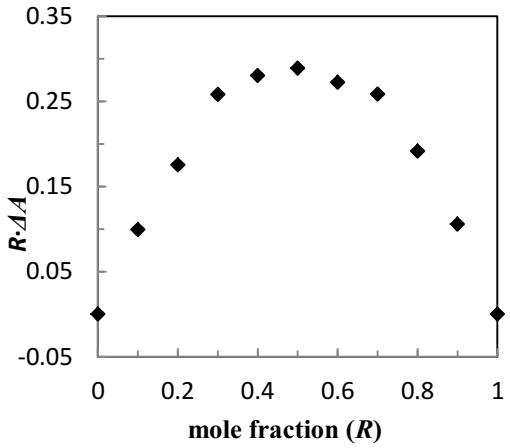
■ **Figure VIII.1.** Plot of surface tension of hydrazinophthalazine as a function of βCD concentration



■ **Figure VIII.2.** Plot of $C_o/(\gamma-\gamma_o)$ of hydrazine-phthalazine against concentration of βCD (C^{-n}; n=1 or 2)



■ **Figure VIII.3.** Jobs plot of hydrazinophthalazine + βCD



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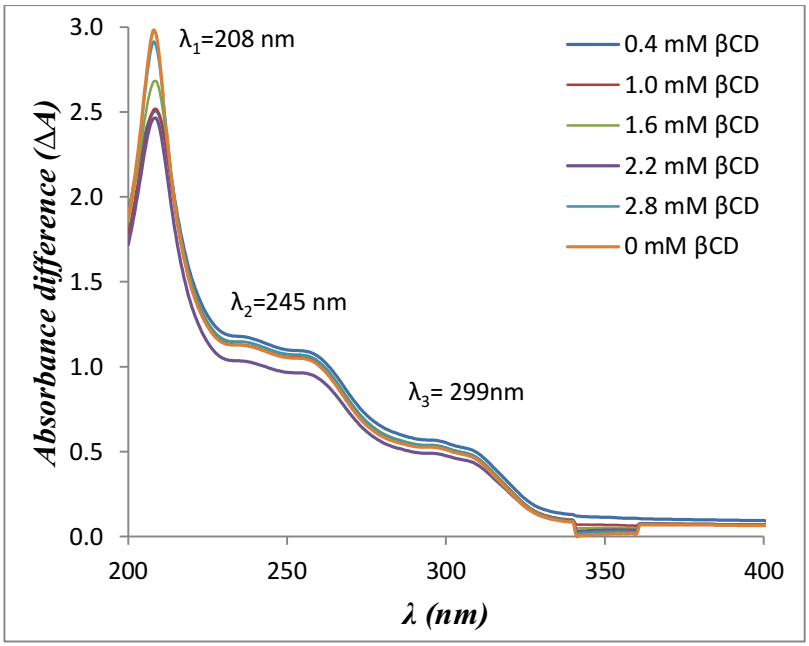
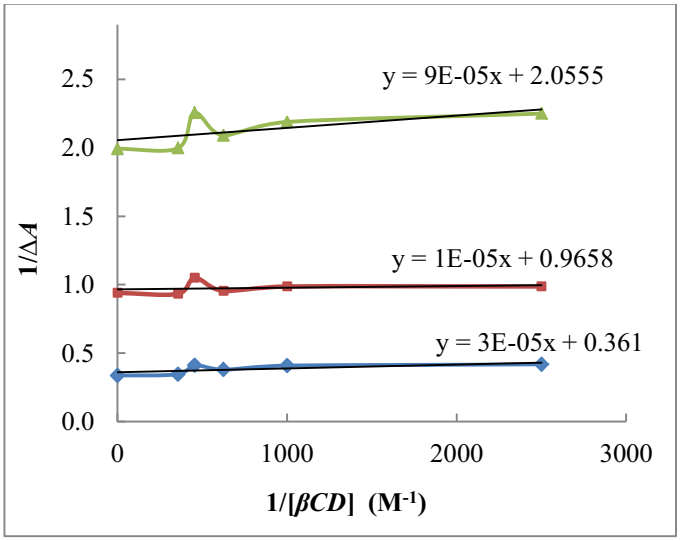
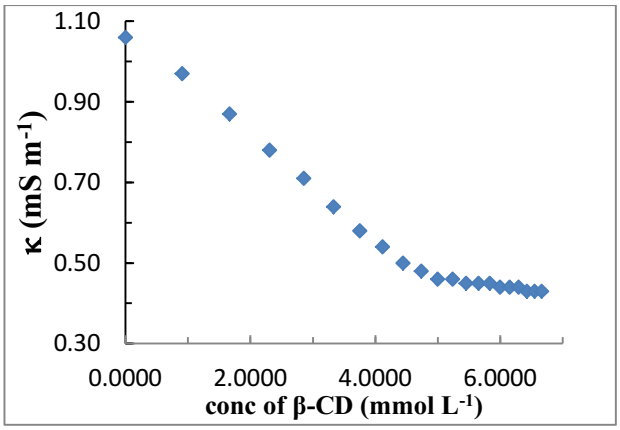
■ **Figure VIII.4.** Absorption spectra of hydrazinophthalazine in presence and absence of βCD



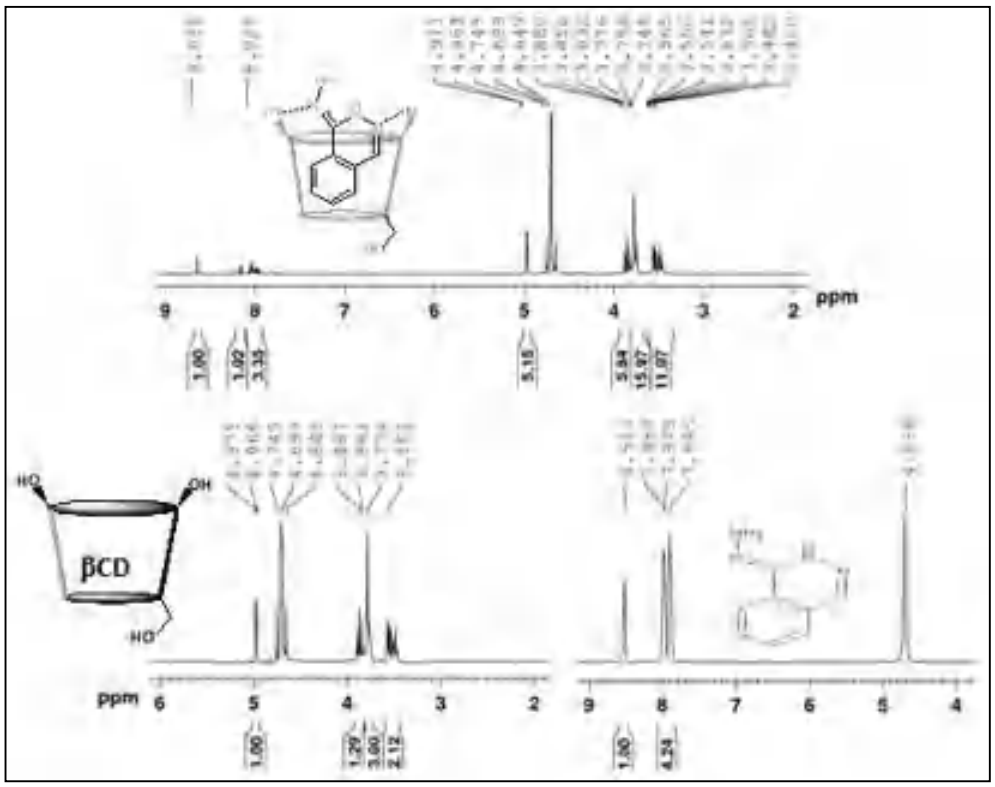
■ **Figure VIII.5.** Double reciprocal plot of $\frac{1}{\Delta A}$ vs $\frac{1}{[\beta CD]}$



■ **Figure VIII.6.** Plot of specific conductivity (κ) corresponding to conc. of β-CD.



■ **Figure VIII.7.** ^{1}HNMR spectra of complex, β-CD, and pure hydrazinophthalazine in D_2O respectively



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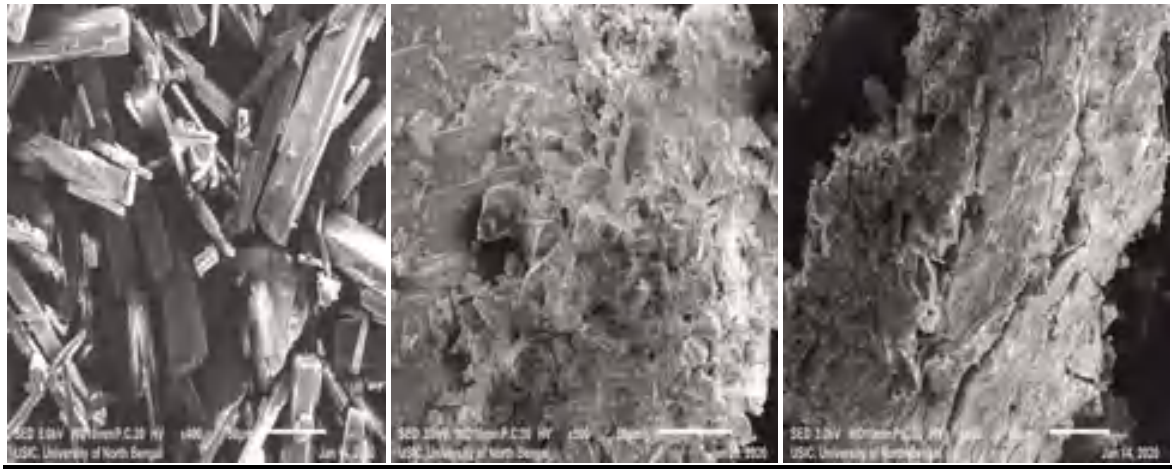
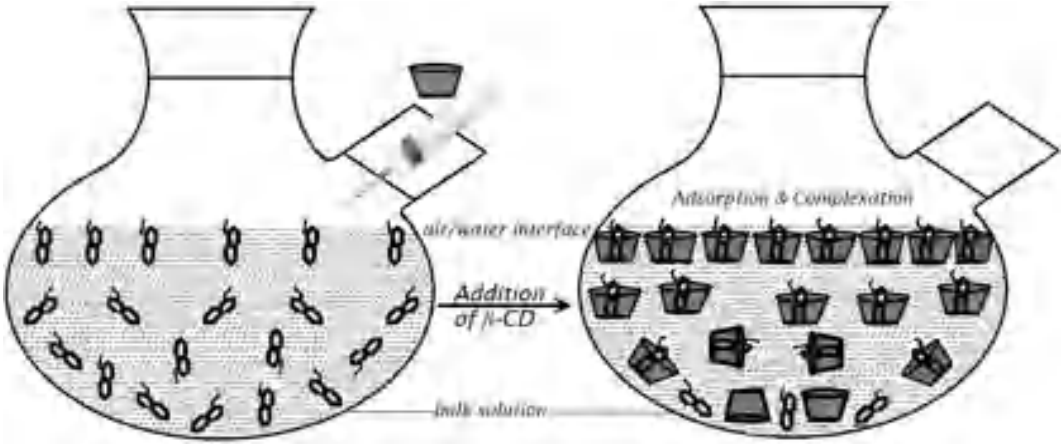
■ **Figure VIII.8.** SEM picture of hydrazinophthalazine, β-CD, and inclusion complex respectively

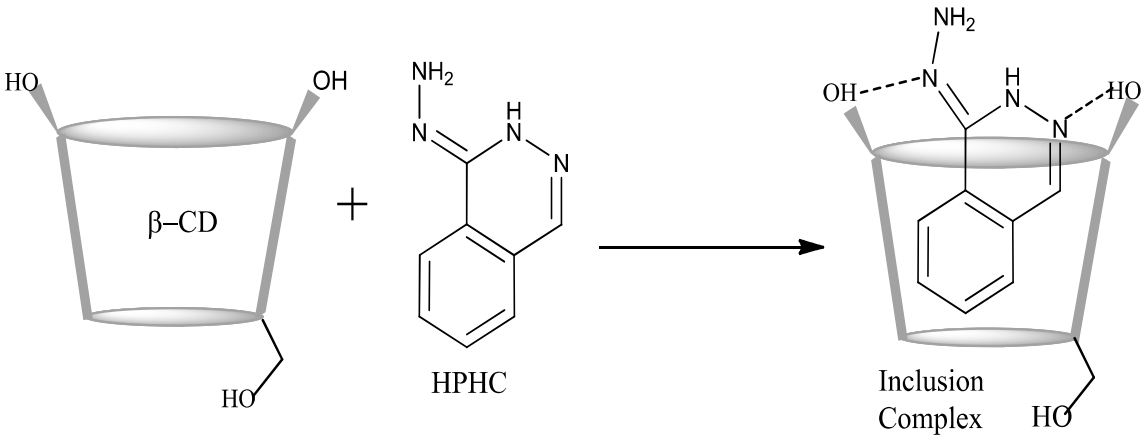


SCHEMES

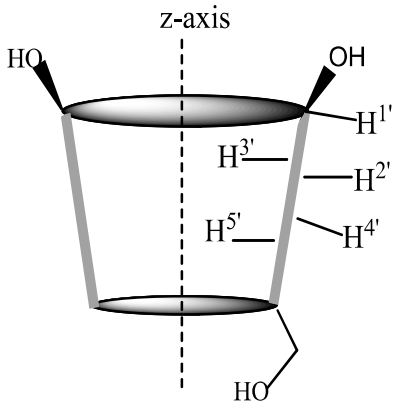
■ **Scheme VIII.1.** Schematic representation of inclusion complex between the hydrazinophthalazine and β-CD in aqueous media



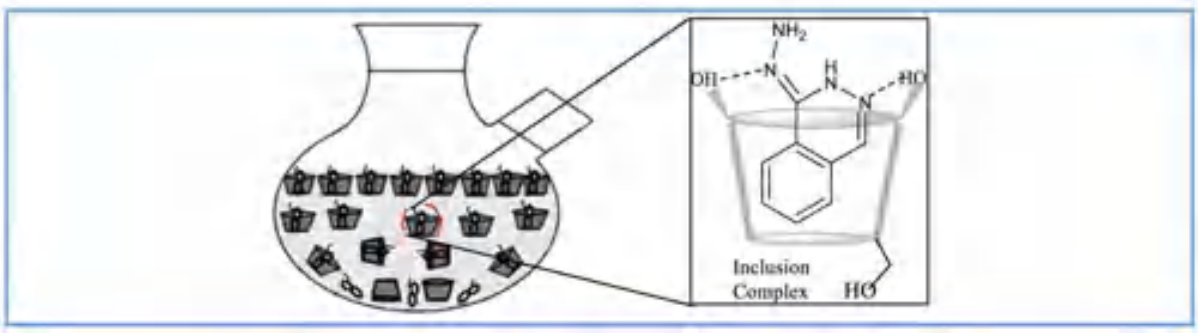
■ **Scheme VIII.2.** Schematic representation of 1:1 inclusion complex



■ **Scheme VIII.3.** Interior and exterior proton of β-cyclodextrin



GRAPHICAL ABSTRACT



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SUPPORTING DATA

■ **Table S1.** Surface tension of hydrazinophthalazine hydrochloride (HPHC)

Conc of βCD (mM)	ln C of βCD	γ (mN·m^{-1})
0.00	0	72.9
0.91	-0.10	73.7
1.67	0.51	74.5
2.31	0.84	75.3
2.86	1.05	75.9
3.33	1.20	76.6
3.75	1.32	77.3
4.12	1.42	77.8
4.44	1.49	78.3
4.74	1.56	78.9
5.01	1.61	79.4
5.24	1.66	79.6
5.45	1.70	79.8
5.65	1.73	80.0
5.83	1.76	80.1
6.00	1.79	80.3
6.15	1.82	80.4
6.30	1.84	80.5
6.43	1.86	80.6
6.55	1.88	80.6
6.67	1.90	80.7

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■ **Table S2.** $C_o/(\gamma-\gamma_o)$ of hydrazinophthalazine against concentration of βCD (C^{-n}; n=1 and 2)

C^{-1} (mmol $L^{-1})^{-1}$	C^{-2} (mmol $L^{-1})^{-2}$	$C_o/(\gamma-\gamma_o)$ (mmol $L^{-1}mN^{-1}$ m^2)
1.100	1.210	12.50
0.600	0.360	6.25
0.433	0.188	4.17
0.350	0.123	3.33
0.300	0.090	2.70
0.267	0.071	2.27
0.243	0.059	2.04
0.225	0.051	1.85
0.211	0.045	1.67
0.200	0.040	1.54
0.191	0.036	1.49
0.183	0.034	1.45
0.177	0.031	1.41
0.171	0.029	1.39
0.167	0.028	1.35
0.163	0.026	1.33
0.159	0.025	1.32
0.156	0.024	1.30
0.153	0.023	1.30
0.150	0.023	1.28

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