CHAPTER IX

PROBING INCLUSION COMPLEX OF N,N-DIMETHYL-4-PHENYLENEDIAMINE DIHYDROCHLORIDE WITH β-CYCLODEXTRIN MOLECULE FOR ENHANCING ITS EFFECTIVENESS IN CHEMICAL AND BIOLOGICAL SCIENCES

Abstract

Inclusion complex forms by the encapsulation of apolar part of N,N-dimethyl-4-phenylenediamine dihydrochloride insight into the hydrophobic cavity of β-cyclodextrin. The complex has been prepared in aqueous medium and characterize by employing several physicochemical and spectroscopic techniques. The origination of (DMPD+β-CD) inclusion complex was established by UV-Visible Job's plot. 1H NMR, FT-IR and SEM studies also account for the inclusion phenomenon. Appraisement of both conductivity as well as surface tension data implies 1 : 1 stoichiometry of the inclusion complex. Association constant and free energy change is 23600 M^{-1} and -24.54 kJ M^{-1} respectively. The formation of the inclusion complex has further accomplished by hydrophobic effects, structural effects, electrostatic forces and H-bonding interactions.

Keywords:

N,N-dimethyl-4-phenylenediamine dihydrochloride; β-Cyclodextrin; Inclusion complex; Physicochemical and Spectroscopic Techniques

1. Introduction

The hollow cylindrical oligosaccharides viz., α-, β- and γ-cyclodextrin [1,2] are truncated conical in structure consist of inner cavity of hydrophobic nature. In contrast, the exterior is sufficiently hydrophilic that impart the cyclodextrins (or its derivatives) water solubility. [3] While, the wider rim has all secondary hydroxyl groups , the narrow one has all primary hydroxyl groups (Scheme IX.1). This particular feature facilitates cyclodextrin to form stable host-guest inclusion complexes through the insertion of hydrophobic part of incoming guest molecules. [4]

The incorporation of hydrophobic part of a guest moiety into the cavity of cyclodextrin is energetically unfavourable. The water molecules in the cavity of cyclodextrin in aqueous medium are the main driving force and create faceable condition for encapsulation. Molecules having appropriate dimension and a hydrophobic moiety can replace the water molecules. The energetically unfavourable water molecules come out from the cavity and pull the guest molecules insight into the cavity; as a result stable complex is formed. This property is excellently utilized to macromolecules, especially for shielding the hydrophibic part of guest molecules from deformation, degradation by auto-oxidation, hydrolysis, proteolysis. [5] For this unique property, cyclodextrins have extensive uses in pharmaceuticals, foodstuffs, pesticides, textile processing and toilet articles.[6,7] The control release ability of CD is also used in cosmetic and paint industries, removing different toxic materials, pollutants and waste products without a chemical change. [8]

N,N-dimethyl-4-phenylenediamine dihydrochloride (DMPD) is a derivative of aniline family (Scheme IX.1). It is effective in the oxidase test to differentiate bacteria by the formation of dye. It was used for the determination of the plasma oxidant in terms of plasma ferric equivalent oxidative potential (PFEOP), as an accelerator for the vulcanization of rubber. [9] Therefore, inclusion of DMPD molecule with β-CD may give rise to a superior application, like synthesis of DMPD derivatives, to protect the N,N-dimethyl part from the reaction, modification of cosmetics, reduce toxicity etc.

In this work, we have focused on the preparation and characterization of the inclusion complex formed by the encapsulation of N,N-Dimethyl-p-phenylenediamine dihydrochloride (DMPD) insight into β-cyclodextrin. Physicochemical properties (surface tension, conductivity measurements) and spectroscopic techniques (UV-Vis, ^{1}H NMR, 2D NMR, FT-IR, SEM picture) have been employed to elucidate the stoichiometry and stability of the complex.

2. Experimental Section

2.1 Materials

N,N-dimethyl-4-phenylenediamine dihydrochloride (CAS Number- 3575-32-4, purity 99%) and β-cyclodextrin (CAS Number- 7585-39-9, purity 97%) of puris grade were purchased from Sigma-Aldrich. The chemicals have been kept in dry and dark place in a vacuum desiccator (with CaF_2 crucible). They are used as received without any further purification.

2.2 Apparatus and procedure

The UV-Visible spectra were recorded utilizing a JASCO V-530 UV–Vis spectrophotometer. The wavelength accuracy was ±0.5 nm and the cell temperature during the experiment was regulated with a digital thermostat.

^{1}H NMR spectra were recorded in D_2O solvent at 400MHz in a Bruker Avance instrument. The residual protonated signal (HDO, δ 4.79 ppm) was used as an internal standard.

By means of JEOL JSM-IT 100 Scanning Electron Microscope (SEM), the surface morphologies of DMPD, β-CD and the complex were recorded. Images were taken at an excitation voltage of 30kV with a magnification of 2000X.

Surface tension of the solutions was measured by platinum ring detachment technique with a K9 digital tensiometer (Krüss GmbH, Hamburg, Germany) at the room temperature. The accuracy of measurement was ±0.1 $mN{\cdot}m^{-1}$.

The METTLER-TOLEDO 7 multi conductivity meter was used for the measurement of specific conductivity with an uncertainty of ±1.0 μS m^{-1}. HPLC-grade water with a specific conductance of 6.0 μS m^{-1} was used for conductivity measurement. The conductivity cell was calibrated using a freshly prepared 0.01M KCl solution.

2.3 Procedure and preparation of the complex

Prior to perform the experiment, the solubility of the chosen cyclodextrin and DMPD were checked in triply distilled, deionized, and degassed water. In order to prepare the solutions the mass measurements were done by Mettler Toledo AG-285 analytical balance with an uncertainty of ±0.1 mg. Adequate precautions were taken to minimise the evaporation and losses during the preparation of complex and working with solutions. In order to prepare the inclusion complex, 1.0 mmol of DMPD and 1.0 mmol of CD were separately dissolved in 20 mL of water and stirred for more than 4h. Afterwards, the DMPD solution was added drop wise to the CD solution and then stirred for 48 hours at 50–55°C. The mixture was then cooled to 5°C for ≈12 hours. Finally, the resulting suspension was filtered and a white polycrystalline powder was obtained. It was then washed with ethanol and dried in air.

3. Results and discussion

3.1 Conductivity and surface tension study

Here, two physicochemical properties viz. conductivity and surface tension have been studied on the ternary solution of DMPD and β-CD in aqueous media. Initially, 10ml 5mM DMPD solution was taken, and then conductivity and surface tension were measured separately upon the sequential addition of β-CD. A gradual fall in conductivity and rise in tensiometric curve were observed (Figure IX.1). Initially conductivity (3.43 μS m^{-1}) and surface tension (61.2 mN m^{-1}) is showing for DMPD. The decreasing trend in conductivity and increasing fashion in surface tension is probably for the trapping of charged DMPD molecules by β-CD. [10,11] This trend takes place till all the DMPD molecules were encapsulated by cyclodextrin. A break in each of the conductivity and surface tension curve has been found at a certain concentration (5.23 mM) of β-CD. After the break point the curve remains nearly parallel with β-CD. The parallel variation

is due to the non-conductive and surface inactive cyclodextrin molecules. The concentration ratio of DMPD and β-CD at the break point (5 mM : 5.23 mM) suggests the formation of 1:1 stoichiometric complex. [12-15]

3.2. UV-Visible Spectroscopy

Job's method, or the continuous variation method has been employed to talk about the stoichiometry of inclusion complexes. [16] UV-Visible spectral data were used in this method. Separately 100μM solutions of DMPD and β-CD were prepared. After that, the mixed solutions of DMPD and β-CD were prepared by varying the molar ratio (*viz.,* 4ml:0ml, 3.6ml:0.4ml, 3.2ml:0.8ml and so on) by keeping the total concentration of the species constant. The absorbance was measured at λ_{max} (237 nm) for all solutions, because at maxima, the π-π* transition of the aromatic moiety occurs. In Figure 2, $R\Delta A$ values were plotted against R, so as to obtain Job plots. Here, ΔA is the difference in absorbance of the DMPD in absence and presence of cyclodextrin; and R = [DMPD]/([DMPD]+[β -CD]).

The value of R at the highest deviation discloses the stoichiometry of the complex. A 1:1 stoichiometry comes out, when R is 0.5; similarly 1:2 and 2:1 ratio comes when the guest is present 0.33 and 0.67 fractions respectively with respect to host (Scheme IX.4). Here, Figure IX.2 shows the curve maxima at R = 0.5. This ratio implies a 1:1 stoichiometry of the inclusion complex . [17]

The Figure IX.3. shows the variation in UV-vis spectra of different strength (20 mM, 50 mM, 80 mM, 110 mM, 140 mM respectively) of DMPD on addition of β-CD. The absoption maxima was observed at 237 nm. For the guest, λ_{max} = 237 nm was considered to determine the binding constant (K_a).

Assessment of binding constant (K_a) values demonstrate both the extent of encapsulation into the host's cavity and also the stability of the complex formed. UV-VIS spectra have been used to find out the binding constant. While forming the inclusion complex, the guest molecule from a polar aqueous media migrates to a non-polar hydrophobic cavity of β-CD. This results in a change in molar extinction coefficient (Δε) of DMPD solution. [18,19] Now, using Benesi-Hindebrand double reciprocal method,

the reciprocal of changes in absorbance (ΔA) were interpreted in terms of the reciprocal of cyclodextrin concentration.

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon\,[DMPD]K_a}\,\frac{1}{[CD]} + \frac{1}{\Delta\varepsilon\,[DMPD]}$$

Plot of 1/ΔA versus 1/[β-CD] was found to be straight line. The binding constant for the complexation can be calculated simply by dividing the slope by the intercept of the plot given in the Figure IX.4. at 20 mM, 50 mM, 80 mM, 110 mM, and 140 mM respectively.

From Table IX.1, it has been found that the binding constant is 23600 M^{-1} and Gibbs free energy is -24.54 kJ mol^{-1}. The results are quite satisfactory and are in agreement with the formation of stable complex. [20]

On the other hand, fitting UV-VIS spectroscopic data into a non-linear program to determine association constant $K_a{}^{\theta}$. [21] The formation of 1:1 complex may lead to the following equilibrium to exist between the guest and host molecule. [22]

$$[DMPD]_f + [CD]_f \rightleftharpoons [IC]$$

Thus, the association constant ($K_a{}^{\theta}$) can be obtained,

$$K_a^{\theta} = \frac{[IC]}{[DMPD]_f\ [CD]_f}$$

Where, [IC], $[DMPD]_f$ and $[CD]_f$ are the equilibrium concentration of inclusion complex, free DMPD and free cyclodextrin respectively. $K_a{}^{\theta}$ can also be expressed in terms of absorbance of the guest and host molecules,

$$K_a^{\theta} = \frac{[IC]}{[DMPD]_f\ [CD]_f} = \frac{(A_{obs} - A_0)}{(A - A_{obs})\ [CD]_f}$$

Here,

$$[DMPD]_f = [CD]_x - \frac{[DMPD]_x\ (A_{obs} - A_0)}{(A - A_{obs})}$$

Where, A_0, A_{obs} and A stands for the initial absorbance of DMPD, absorbance during the gradual addition of CD and final concentration of DMPD. $[DMPD]_x$ and $[CD]_x$ are respectively the concentrations of guest and the host added.

3.3 ^{1}H NMR and 2D NMR Spectral Analysis

Formation of inclusion complexes can also be illustrated by ^{1}HNMR spectroscopic study. [23] The study also affords the explication of the probable mode of inclusion as well as the quantitative information on spatial arrangement of the guest with the CD molecule. [24] The ^{1}H NMR of β-CD, DMPD along with the inclusion compound they form upon complexation, have been studied and shown in the Figure IX.5. The host's cavity exerts a hydrophobic environment to the encapsulated guest molecule, very much different from that of the bulk solution. [25,26] This results in an appreciable change in chemical shift values of different protons of both the guest and the host. A close look at the structure of cyclodextrin molecule reveals that the H3 and H5 protons are situated within the conical cavity. Moreover, the H3 are placed near the wider rim whereas H5 are located at the proximity of narrower rim of cyclodextrin molecule but the H1, H2 and H4 protons are placed at the periphery of the β-CD molecule (Scheme IX.2). [27, 28] Inclusion of the guest moiety DMPD into the cavity of the host, leads to an increase in electron density over the H3, H5 protons thereby resulting into shielding of the protons to induce an up field shift. [29] The up field shift of these protons along with the significant shift of the interacting aromatic protons of DMPD molecule clearly implies the presence of DMPD molecule inside the CD cavity. The greater change in chemical shift (δ) of the H3 hydrogen compare to that of the H5 hydrogen confirm that the guest entered through the wider rim of β-CD. [30] Apart from these, negligible chemical shifts were also observed for the H1, H2 and H4 protons, which are not the part of the hydrophobic cavity of β-CD.

Two-Dimensional NMR (2D NMR) spectroscopy offers critical evidence regarding the spatial vicinity of the interacting atoms of the host guest duo by observing the intermolecular dipolar cross-correlations. [31,32] When two protons are located within a distance of 0.4 nm in space, they can generate a nuclear overhauser effect (NOE) cross-correlation in NOE spectroscopy (NOESY) or rotating-frame NOE spectroscopy (ROESY). [33] The inclusion of guest molecule into the cavity of β-CD can be established

by the appearance of NOE cross-peaks between the H3 or H5 protons of CD and the interacting protons of the guest molecule disclosing their proximity in space. [34,35] The 2D ROESY spectra obtained for the inclusion complex of DMPD and β-CD in D_2O, also showed substantial correlation between the aromatic protons of DMPD and the H3 and H5 protons of the host (Figure IX.6.). This signify the encapsulation of aromatic ring inside the CD cavity. [36] The fact that the H6 protons of β-CD molecule remain uninfluenced by the inclusion procedure yet again suggest that the incorporation of DMPD molecule into the host's cavity occurs through the wider rim, but, not through the narrower rim, because, in that case, cross-peaks between the H6 proton and the guest would have been obtained. [37]

3.4 SEM analysis

Scanning Electron Microscopy (SEM) is another proficient technique for the exploration of the change in surface texture and particle size of host and guest compounds upon complexation. [38,39] The SEM images exhibiting surface morphological structures of DMPD, β-CD, and (DMPD+β-CD) complex are shown in Figure IX.7. From this Figure, it is evident that all the morphological structures are quite different from each other. Indeed, the original morphology of DMPD disappeared almost completely, and it was also impossible to differentiate the two components of DMPD and β-CD, in the inclusion complex. This might be regarded as an additional testimony for the formation of inclusion complex between DMPD and β-CD, as evident from UV-Vis, ^{1}H NMR analysis as well.

3.5 Structural influence of host and guest

Since the hydrophobic cavity and hydrophilic rims of cyclodextrin molecule provides a suitable environment for the apolar part of a competent guest to be situated inside the cavity; whereas, the polar portion gets associated with the polar rims. These give rise enormous stability to the inclusion complex thus formed. [1] The qualified dimension of apolar benzene ring in DMPD is 5.25 Å. This width is smaller than the diameter of the inner cavity of β-CD (6.0 - 7.0 Å). [40] The dimensional fit of DMPD and β-CD, binds them effectively and form 1:1 inclusion complex. However, the formation of complex is not associated with breaking or making of any covalent bond. [41] They binds together

with non-covalent bonds like H-bonds, hydrophobic-hydophobic interaction, hydrophilic-hydrophilic interaction etc. (Scheme IX.3). Both the polar amine groups in DMPD molecule projected outside the rims of β-CD involves in H-bond formation with the –OH groups at the rims of CD molecules thereby stabilizing the complex.

4. Conclusion

This comprehensive study describes the inclusion phenomena of N,N-dimethyl-4-phenylenediamine dihydrochloride with β-Cyclodextrin. 1:1 inclusion complex formed between DMPD and β-CD. The solid complex was found to be freely soluble in water. The stoichiometry of the complex was established by UV-Vis, conductance and surface tension study. ^{1}HNMR data reveal the mode of inclusion where the hydrophobic aromatic part of the guest molecule was incorporated. The reliable value of association constant and free energy stands for the stability of the inclusion complex. The complaxation is occurring by the binding between DMPD and β-CD with non-covalent bonds, where H-bonding interactions played a significant role.

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Conflict of interest

All the authors declare no conflicts of interest.

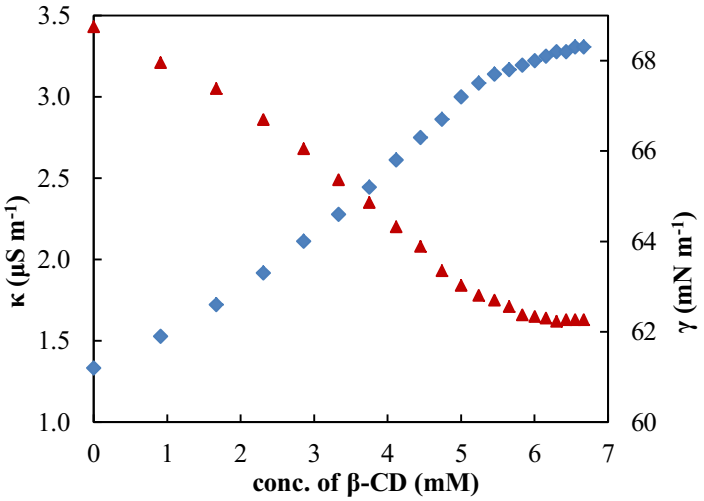
TABLES

■ **Table IX.1.** Association Constant and change in free energy for the (DMPD+β-CD) complex

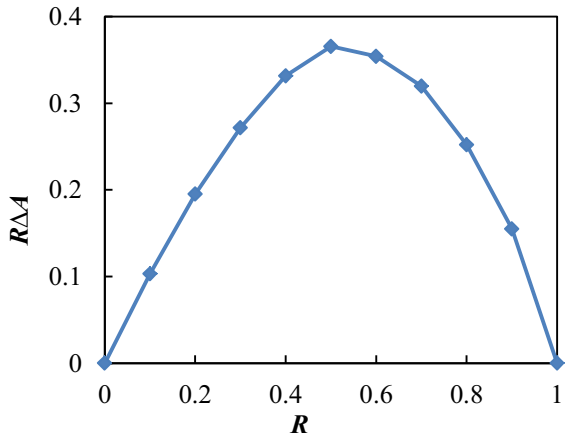
System	T (K)	Slope	Intercept	K_a (M^{-1})	ΔG (KJ mol^{-1})
DMPD+β-CD	293.15	1494.6	35.313	23600	-24.543

FIGURES

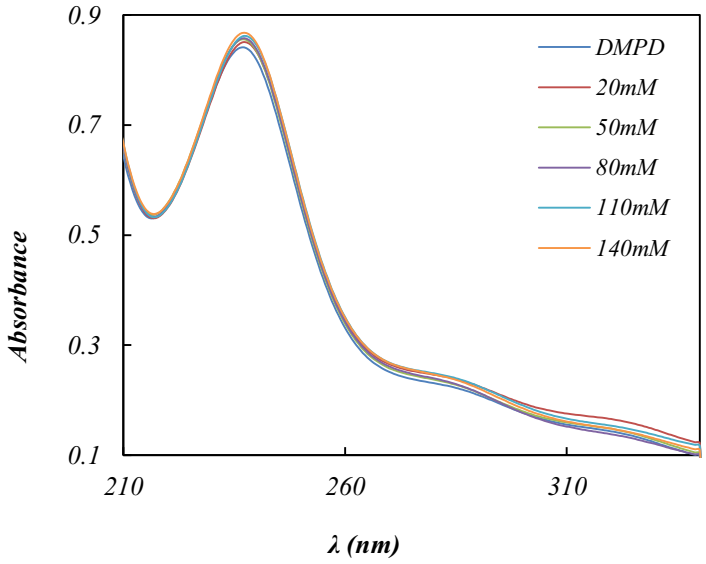
■ **Figure IX.1.** Variation of specific conductivity (κ) and surface tension (γ) of DMPD with concentration of β-CD



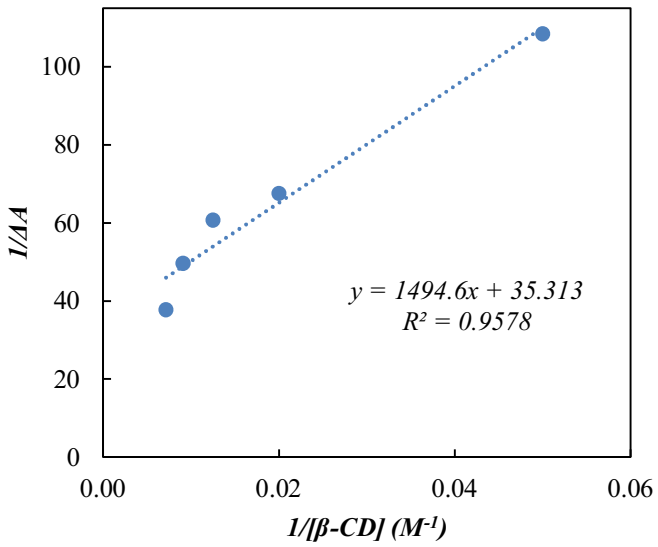
■ **Figure IX.2.** Job's plot of DMPD with β-CD



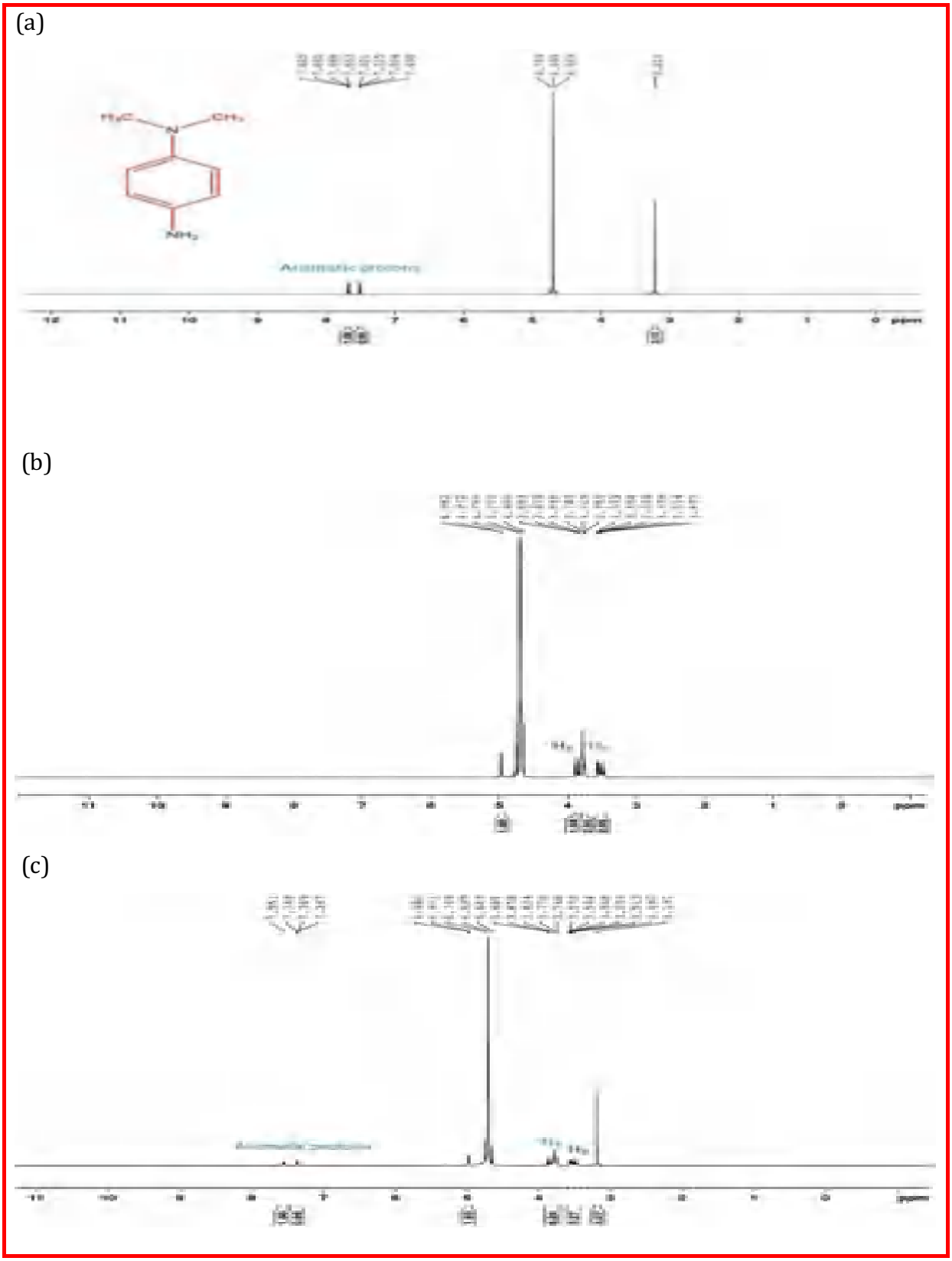
■ **Figure IX.3.** UV-vis spectral changes on addition of β-CD



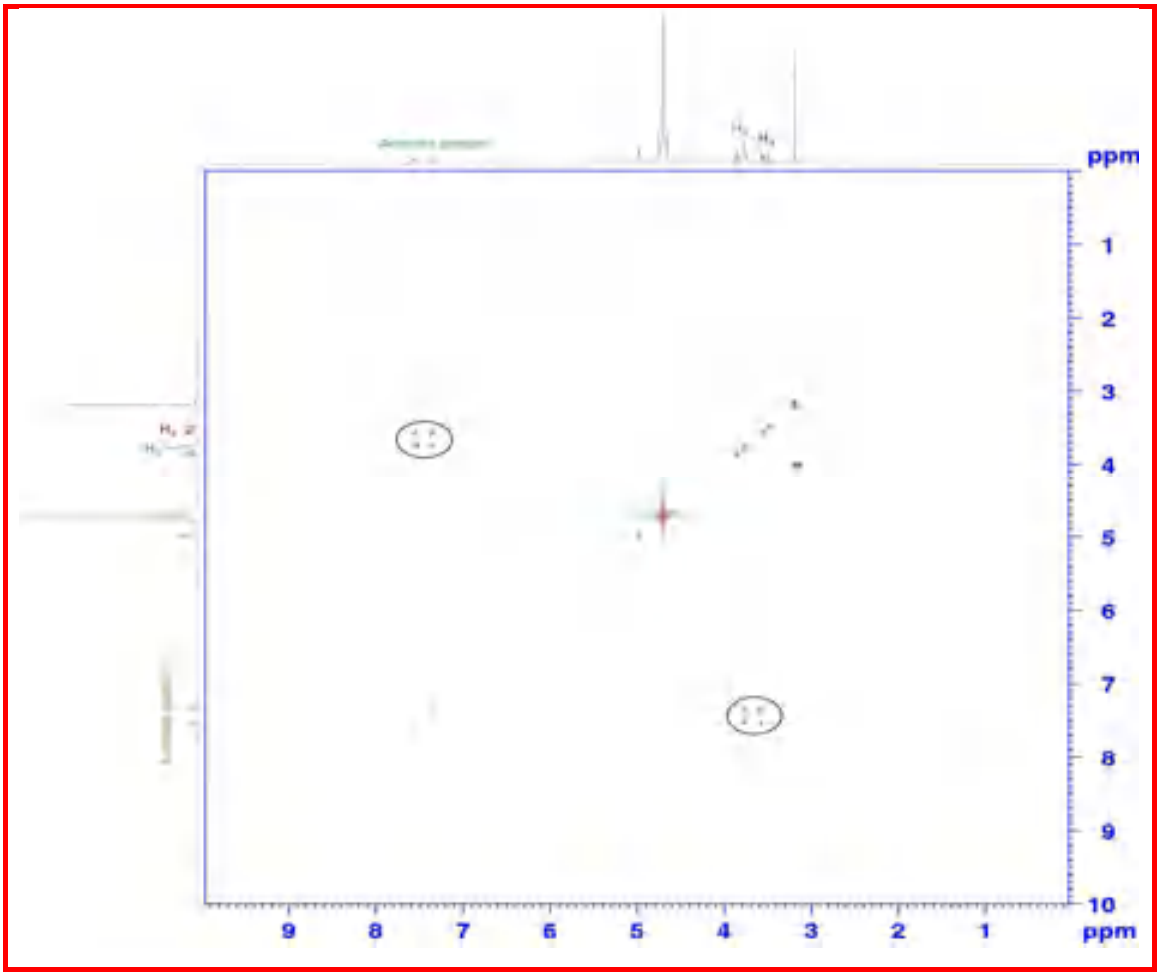
■ **Figure IX.4.** Linear plot of *1/ΔA* vs 1/[β-CD] for DMPD+β-CD system



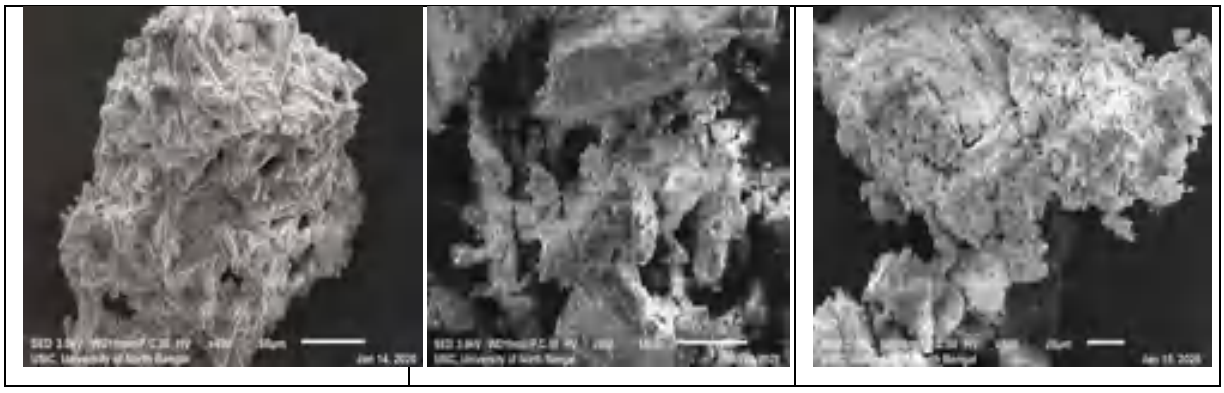
■ **Figure IX.5.** ^{1}HNMR spectra of pure (a) DMPD, (b) β-CD and (c) inclusion complex between DMPD and β-CD (in D_2O, 400 MHz)



■ **Figure IX.6.** 2D ROESY spectra of the solid inclusion complex of DMPD and β-CD

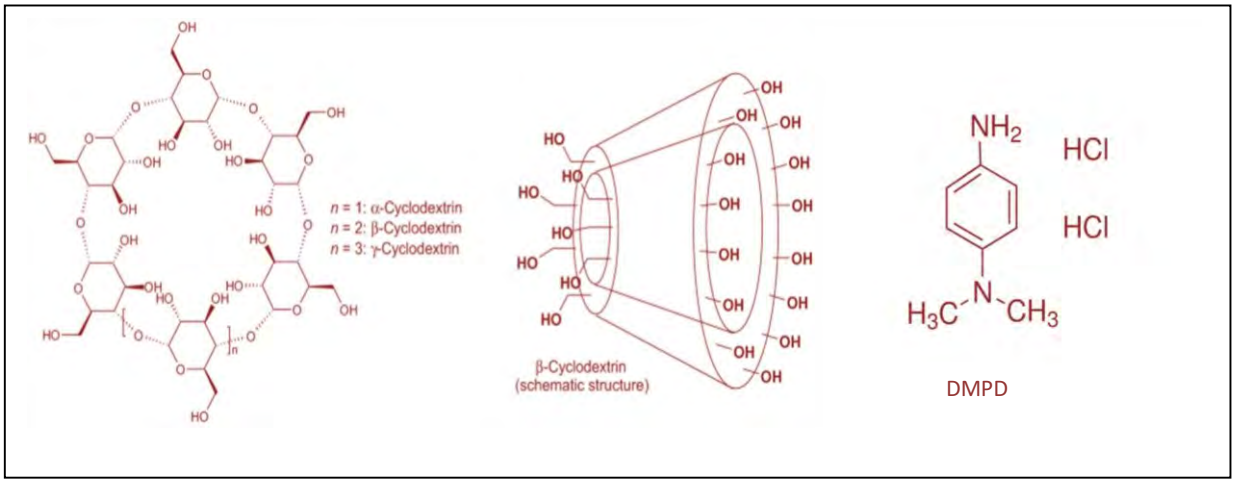


■ **Figure IX.7.** Scanning electron photograph for (a) DMPD, (b) β-CD, (d) (DMPD+β-CD) inclusion complex

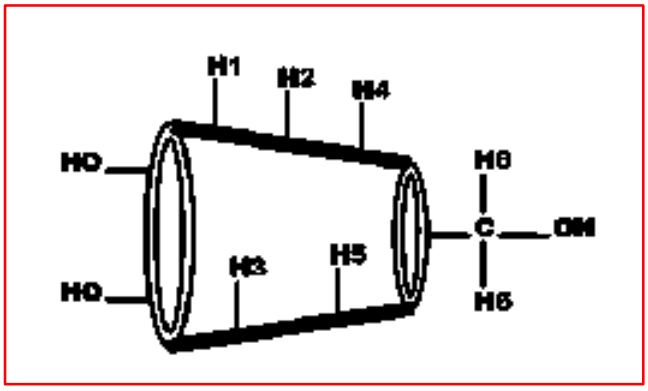


SCHEMES

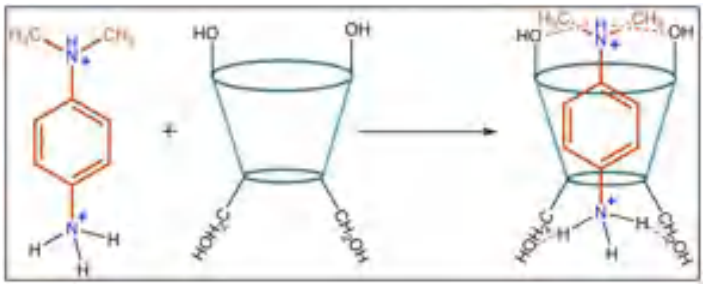
■ **Scheme IX.1.** Molecular structure of β-CD and DMPD



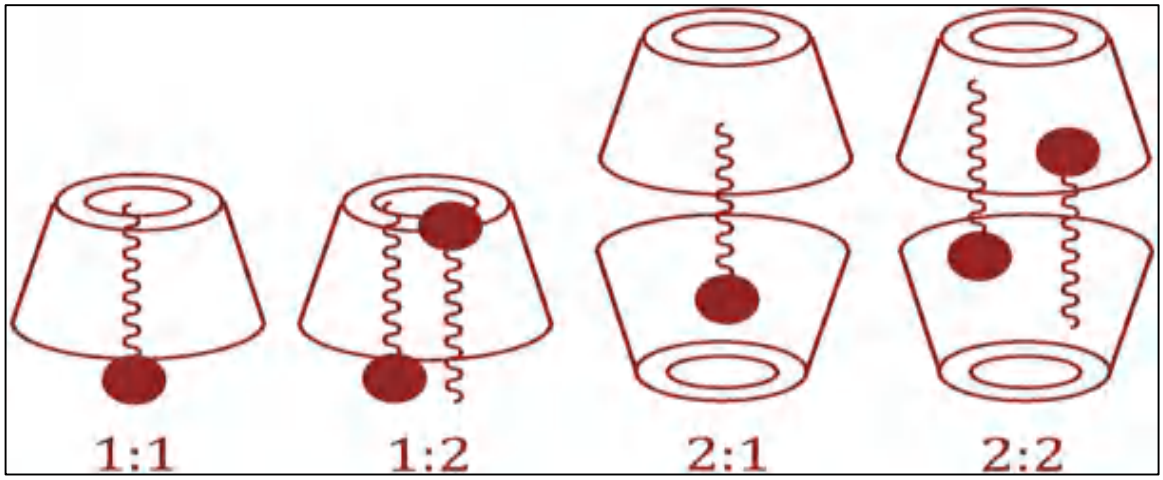
■ **Scheme IX.2.** Molecular structure of β-Cyclodextrin molecule with interior and exterior protons



■ **Scheme IX.3.** Plausible mechanism of the inclusion complaxation



■ **Scheme IX.4** Probable host:guest stoichiometric ratio of the inclusion complexes



SUPPORTING INFORMATION

■ **Table S1.** Values of Surface Tension (γ) and Conductivity (κ) at the Break Point with Corresponding Concentrations of DMPD and β-CD at 298.15 K[a]

Conc.of DMPD (mM)	Conc. Of β-CD (mM)	γ^a (mN m^{-1})	K^a (mS m^{-1})
5.23	4.77	67.5	1.78

[a]Standard uncertainties (u): temperature: u(T) = ±0.01 K, surface tension: u(γ) = ±0.1 mN m^{-1} , conductivity: u(κ) = ±0.001 mS m^{-1} .