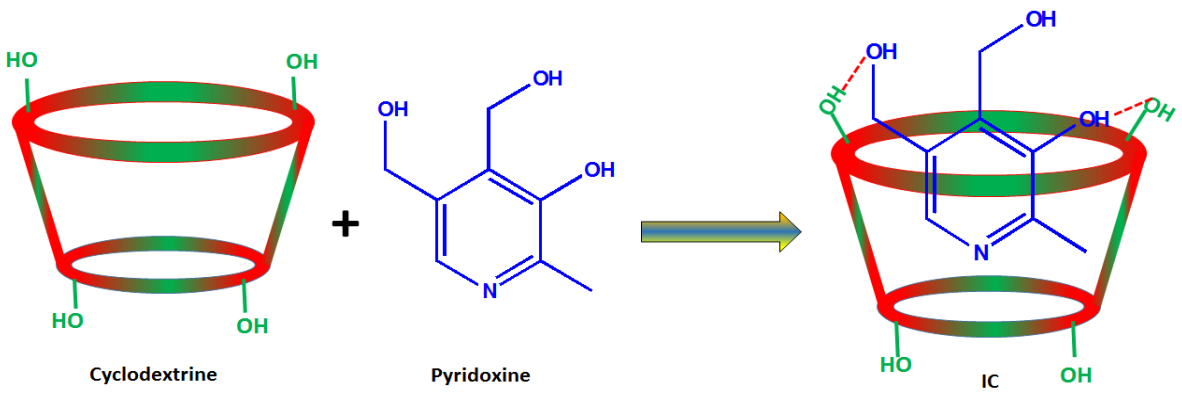
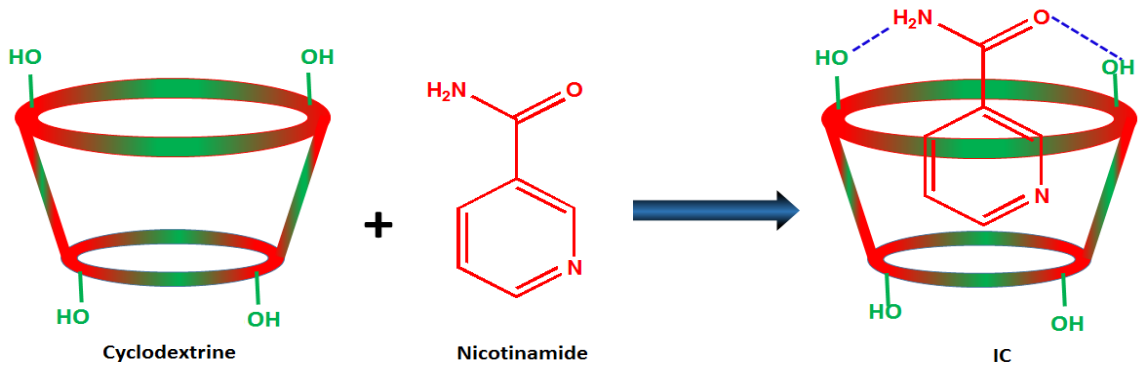
CHAPTER-X

Synthesis and Characterization of Inclusion Complexes of Vit B_3 and Vit B_6 to Explore their Applications in Drug Delivery Systems

Highlights ⇒ Study of Vitamin B complexes with β-CD is portrayed in the recent works along with its controlled delivery in pharmaceutics.

Schematic representation





Abstract

In the present study, Host–guest inclusion complexes of *β*-cyclodextrin with two active forms of vitamins *viz.*, Nicotinamide(vitamin B3) and Pyridoxine(vitamin B6) in aqueous medium have been explored by reliable spectroscopic, physicochemical and *in Vivo* toxicity and *in*

Vitro anti inflammatory experiments as stabilizer, carrier and regulatory releaser of the guest molecules. Job's plots obtained by UV-visible spectroscopy to confirm the 1:1 stoichiometry of the host-guest assembly. Stereochemical nature of the inclusion complexes has been elucidated by NMR spectroscopy. Surface tension and conductivity studies further shore up the inclusion process. Association constants for the vitamin-*β*-CD inclusion complexes have been calculated by UV-visible spectroscopy using Benesi–Hildebrand methods, non-linear programme, and Stern-Volmer approximation technique while the thermodynamic parameters have been estimated with the help of van't Hoff equation. The outcomes reveal that there is a drop in ΔSo, which is overcome by higher negative value of ΔHo, making the overall inclusion process thermodynamically favorable. The association constant is found to be higher for Pyridoxine than that for nicotinamide, which has been explained on the basis of their molecular structures.

We also reported experimental densities and viscosities of aqueous solutions of Nicotinamide and Pyridoxine within the concentration range (0 to 0.1) mol. $kg^{-}1$ at diverse temperatures. These parameters are thus used to provide thermodynamic and transport functions such as apparent molar volume of solute, limiting apparent molar volume of solute, limiting apparent molar expansivity of solute, coefficient of thermal expansion, Jones–Dole equation viscosity A, B and D coefficients, temperature derivative of B coefficient i.e. (dB/dT) and hydration number, etc. Activation parameters of viscous flow for the binary mixtures have been determined and discussed in terms of Eyring's transition state theory. These significant parameters are supportive to study the structure making or destroying tendency of solute and assorted interactions present in (Nicotinamide + β-CD ─ water) and (Pyridoxine +β-CD─ water) ternary mixtures. Various results revealed that the solutions are characterized predominantly by solute-solvent interactions and vitamins behave as a long-range structure maker.

Introduction

Cyclodextrins are toroidally fashioned polysaccharides comprised usually of six to eight glucose units. Cyclodextrin cavities comprises internal diameters ranging from 4.7 to 8.3 A, permitting them to form inclusion complexes with a variety of guest molecule. They have been used extensively to model hydrogen bonds, π–π stacking, hydrophobic interactions, and metal–coordination bonds interactions. The most important property of CD's is their ability to

admit a variety of selective and specific sized guest molecules into the cavity with formation of inclusion complexes. The recognized potential of CD-guest interaction as a replica for enzyme active sites has engrossed the attention of many investigators. Studies relating inclusion of active pharmaceutical substances into CDs are important due to the resulting improvement of aqueous solubility, stability of guest molecule and to the possibility of controlled drug release, which present lots of potential applications in drug formulations.

Vitamins are indispensable for average growth and development of multicellular organisms. This investigation comprises of two Vitamins, which are being used as the guest moiety in the inclusion complex formation.

Pyridine-3-carboxamide (nicotinamide), also known as Vitamin PP (pellagra protective), Vitamin B3 etc., plays a fundamental role in biological oxidative chemistry. Nicotinamide is active form of amide of nicotinic acid. Nicotinamide belongs to the category of water-soluble vitamin. In cells, niacin is incorporated into nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), although the pathways for nicotinamide and nicotinic acid are analogous. Within the cells, NADP/NADPH performs comparable chemical functions. NAD^+ and $NADP^+$ are coenzymes in a broad variety of enzymatic oxidation–reduction reactions. Nicotinamide possibly toxic to liver at doses exceeding 3 g/day for adult persons. Nicotinamide has verified anti-inflammatory actions which may be of benefited in patients having inflammatory skin conditions like Pellagra and various skin problems like acne vulgaris, rosacea, autoimmune bullous and the compound can suppress antigen induced-lymphocytic transformation and inhibit of 3-5 cyclic AMP phosphodiesterase. Nicotinamide has demonstrated its capacity to block the inflammatory actions of iodides known to precipitate or exacerbate inflammatory acne. Animal studies demonstrate nicotinamide has anti-anxiety (anxiolytic) property. It may well work in a way similar to benzodiazepines. Nicotinamide, or Vitamin B3, prevents immuno suppression caused by UVA and UVB radiation, and may possibly be added to sunscreen. Nicotinamide erstwhile reported to be an effective skin whitener in topical application. Therefore, the structure of nicotinamide has been the subject matter of various assorted experimental and theoretical studies. The amide group in nicotinamide can adopt a variety of tautomeric and rotameric structures in addition to form fascinating molecular associations via hydrogen bonding in the host-guest complexation.

Vitamin B6 consists of three interrelated pyrimidine vitamer derivates: pyridoxine, pyridoxal, and pyridoxamine, and their phosphate esters. Pyridoxine was first isolated in the year 1934 and named by Albert Szent-Gyorgy. The standard B6 vitamin supplement is pyridoxine hydrochloride (HCl), is least expensive to manufacture commercially. All B vitamins assist the body to convert food (carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins, often referred to as B-complex vitamins, also facilitate the body metabolize fats and protein. B-complex vitamins are needed for healthy skin, hair, eyes, liver and the nervous system function properly. All B vitamins are water soluble, and stable in heat and acid mediums, while it is unstable in alkaline solutions and light which implicates that the body does not store them. Vitamin B6 aids the body make several neurotransmitters, chemicals that carry signals from one nerve cell to another in a controlled way with the help of cyclodextrins. It is needed for normal brain development and function, to build the hormones serotonin and nor epinephrine, which influence mood, and melatonin, which helps regulate the sleep and wakefulness. It is rare to have a significant deficiency of B6, even though studies indicate many people may be mildly deficient, especially children and elderly. Symptoms of serious deficiency include: Muscle weakness, Nervousness, Irritability, Depression, Difficulty concentrating, Short-term memory loss, Heart disease, Nausea and vomiting during pregnancy, Age-related macular degeneration, Depression, Premenstrual syndrome, Carpal tunnel syndrome, Rheumatoid arthritis, Tardive dyskinesia.

The physiological and biochemical aspects of the aforesaid vitamins have been studied in detail. Conversely, it has been found that physicochemical data on vitamins is minimal. At low temperatures Cyclodextrin-water jointly is in a highly ordered state, especially at T/K = 298.15. Maximum density is exhibited as a function of temperature, which may be due to a change in equilibrium ratio of the polymeric low density structured form to the monomeric high density unstructured form; in the neighborhood of temperature of maximum density, nature and size of guest molecules plays a very important role in affecting the structure of (water + cyclodextrins) rather than that observed at higher temperatures. Systematic work has been done on Partial molar volumes, compressibility, volumetric properties have been reported at different temperatures. A considerable amount of work has been done on thermodynamic and transport properties of aqueous solutions of nicotinamide and pyridoxine. But all these measurements have been stated at higher temperatures. So the objective of this investigation is to discuss (solute + solute) and (solute + solvent) interactions

amongst (nicotinamide + cyclodextrin + water) and (pyridoxine + cyclodextrin + water) ternary systems. Hence it would be interesting to study how these biologically imperative vitamins affect the highly structured (cyclodextrin + water) co-solvent at various temperatures.

2. Experimental details (Materials and methods)

The compound under investigation namely nicotinamide, pyridoxine and β-cyclodextrin are purchased from Sigma–Aldrich chemicals, USA which are of spectroscopic grade and hence used for recording the spectra as such without any further purification. The solutes were dried in a vacuum oven and were kept in vacuum desiccators over anhydrous fused calcium chloride for more than two days and used without further purification. All the solutions were all set in doubly distilled water on a molality basis using Mettler Toledo AG-285 with having accuracy of ±0.1 mg and uncertainty 0.0001 g.

Densities (ρ) of the solutions were calculated with an Ostwald-Sprengel type pycnometer having a bulb volume of 25 cm^3, internal diameter of the capillary of ~0.1 cm. The instrument was calibrated at 298.15, 308.15, and 318.15 K with doubly distilled water. The pycnometer with test solution was equilibrated in a water bath maintained at (0.01 K of the desired temperature by means of mercury in glass thermo regulator, and absolute temperature was determined by platinum resistance thermometer and Muller Bridge. The pycnometer was then removed from thermostatic bath, properly dried, and weighed. Evaporation losses remained insignificant during actual measurements. Average of triplicate measurements was taken into account. Density values are reproducible to ($\pm$ 3 × 10-5 g. cm^{-3}).

Solution viscosity (η) and relative viscosities was measured through suspended Ubbelohde type viscometer, calibrated by means of triply distilled water, purified methanol, and dry air with dryer. A thoroughly cleaned and perfectly dried viscometer filled with experimental solutions was positioned vertically in a glass walled thermostat (Bose Panda Instruments Pvt. Ltd.) maintained to ±0.01 K. After attaining thermal equilibrium, efflux times of flow were recorded with the aid of stop watch. Flow times were adjusted to ±0.1 s. In all case three repetitions of each data reproducible to ±0.1 s were taken to average the flow times relative viscosities of vitamins in the concentration range (0 to 0.1) $mol.kg^{-1}$ at three different temperatures, viz., T = (298.15, 308.15 and 318.15) K has been measured. The

temperature of the experimental bath was maintained constant up to ±0.002 K by circulating coolant liquid.

Relative viscosity (η_r) values agreed well with the literature with an uncertainty ±1%.

3. Data processing

3.1 Densitometry: group contribution between guest-host in conjunction with **Viscometric measurement: order of contributions.**

In our current study several expensive information about the inclusion phenomenon between the vitamins and CD molecules has been obtained applying density measurement. Apparent molar volume (ϕ_v) and limiting apparent molar volume (ϕ_v^o) are the two major parameters used for this goal. Apparent molar volume (ϕ_v) mainly describes the summation of the geometric volume of central solute (i.e. guest molecule and changes in the solvent volume (i.e., CD along with water) as a result of interface with the solute around the co-sphere (here, solute = nicotinamide, pyridoxine and co-solvent = CD along with water). For this system (ternary phase of vitamin + aqueous CD system) solute-solvent interaction is conveyed by limiting apparent molar volume. For this study (ϕ_v) have been calculated from the density of solution systems by means of equation

$$\rho = W/ V_p - \Pi r^2 dh \qquad (1)$$

where, W is weight of solution, V_p is volume of pycnometer at the aforesaid temperature, r is radius of capillary of pycnometer used and dh is the total height.

The apparent molar volume of solute (ϕ_V) at diverse temperatures is calculated by using the following relation,

$$\phi_V = [1000(\rho_0 - \rho) / m.\ \rho.\ \rho_0] + M_2 /\rho \qquad (2)$$

where (ρ_0) is the density of (water+βCD), M_2 is the molar mass of solute and m is the molality of solution. The limiting apparent molar volume of nicotinamide (1:1 electrolyte) at infinite dilution (ϕ_V^0) was obtained by smooth extrapolation of ($\phi_V - A_V (m)^{1/2}$) against m to zero concentration by using Redlich–Meyer equation [1].

$$\phi_V = \phi_V^0 + A_V . m^{1/2} + S_V m \qquad (3)$$

where A_V is the Debye–Huckel limiting slope which depends upon valency and temperature and it is calculated by the relation,

$$A_V = k_w^{3/2} \qquad (4)$$

where k is the coefficient which is given by the Redlich–Meyer polynomial equation in terms of temperature t/^{0}C [2].

$$k = 1.4447 + 1.6799 * 10^{-2} (t/^0C) - 8.4055 * 10^{-6} (t/^0C)^2 + 5.5153 * 10^{-7} (t/^0C)^3 \quad (5)$$

and w is the valency factor which is determined by the relation

$$w = 0.5\, v_i z_i^2 \quad (6)$$

where v_i is the number and z_i is the charge on each ion of the electrolyte. Using equations (4)–(6) Debye–Huckel limiting slopes A_V for aqueous binary system of nicotinamide at T = (298.15, 303.15 and 308.15) K, are calculated as (1.800, 1.882, 1.970) $cm^3 . mol^{3/2} . dm^{3/2}$ respectively. S_V is the experimental slope of $\{(\phi_V/ - A_V (m)^{1/2}) - m\}$ curve.

When K_d is the dissociation constant, then, it serves as an indicator of the electrolyte strength, strong electrolyte have higher K_d value as in pyridoxine due to two basic centres –O-, -N- and conversely, nicotinamide is a weak electrolyte with dissociation constant $K_d = 2.24 * 10^{-11}$, [3, 4] hence it is treated as non-electrolyte and its limiting apparent molar volume was obtained by smooth extrapolation of (ϕ_V) against m to zero concentration.

$$\phi_V = \phi_V^0 + S_V m \quad (7)$$

Limiting apparent molar expansivity of solute (ϕE^0) has been obtained at T = (298.15 to 308.15) K by using following expression,

$$\phi_E^0 = (d\phi_V^0/dT) \quad (8)$$

The temperature derivative of (ϕE^0) has been calculated at T/ K = 303.15 which is given by the following relation,

$$(\delta\phi E^0/\delta T) = (\delta^2 \phi_V^0/ \delta T^2) \quad (9)$$

The coefficient of thermal expansion of solute (α^*) is given by the following relation,

$$\alpha^* = (1/ \phi_V^0).\ (\delta\phi_V^0/\delta T). \quad (10)$$

The relative viscosity (η_r) of aqueous binary solutions of both the vitamins at all three studied temperatures has been determined by using the following expression,

$$\eta_r = \eta/\eta_0 = t\, \rho/ t_0\, \rho_0 \quad (11)$$

where (η) and (η_0) are viscosities of solution and (water+βCD) respectively, (t) and (t_0) are the flow times for solution and (water+βCD) respectively and (ρ) and (ρ_0) are densities of solution and (water+β CD) respectively.

Viscosity data have been analysed by using the Jones–Dole viscosity equation, [5]

$$\eta_r = 1 + A(C)^{1/2} + BC + DC^2 \quad (12)$$

where A, B and D are Jones–Dole viscosity coefficients and C is molarity of solution. For pyridoxine as an electrolyte, A, B and D coefficients have been determined by plotting ((ηr - 1)/ (C) ½) verses (C) ½.

Since nicotinamide is a non-electrolyte, A = 0 and equation (12) is reduced to

$$\eta_r = 1 + BC \qquad (13)$$

The viscosity B coefficient has been obtained by plotting ($\eta_r - 1$) against molarity (C).

The hydration numbers (η_H) at T/K = (298.15, 303.15 and 308.15) have been determined as,

$$\eta_H = (V^0_e - \phi_V^0) / V_{(\beta CD + H2O)} \qquad (14)$$

where V^0e is the limiting value of the effective volume of the flowing solution, $V_{(H2O+\beta CD)}$ is the molar volume of (water+βCD) and ϕ_V^0 is the limiting apparent molar volume of solute. V^0e is determined by smooth extrapolation of effective volume of flowing solution, V_e to zero concentration C and V_e is obtained by using the Breslau–Miller equation [6], which is derived from the Thomas equation [7].

$$V_e = [-2.5C + \{(2.5C)^2 - 4(10.05C^2)(1 - \eta_r)\}^{0.5}] / 2(10.05C^2) \qquad (15)$$

The values of the standard deviation (σ) and average absolute deviation (AAD) for apparent molar volume of both solutes have been obtained by using the following expression.

$$\sigma = \{\sum (F(x)_{exp} - F(x)_{calc})^2 / (k - n)\}^{1/2} \qquad (16)$$

$$AAD = \{\sum |[F(x)_{exp} - F(x)_{calc}] / F(x)_{exp}|\} / k \qquad (17)$$

where k is the number of experimental points excluding the end points and n is the order of the polynomial equation.

The Gibbs free energy of activation per mole of solvent ($\Delta\mu_1^{0\#}$) is calculated by using the following expression suggested by Feakins et al. [8],

$$\Delta\mu_1^{0\#} = RT. \ln (\eta_0 \tilde{V}_1^0 / h N_A) \qquad (18)$$

where h is Planck's constant, N_A is Avogadro's number, η_0 is the viscosity of (water+ βCD) initially and $\tilde{V}_1^0$ is the molar volume of solvent at that temperature.

Similarly the Gibbs free energy for activation per mole of solute ($\Delta\mu_2^{0\#}$) is calculated by using the value of B coefficient [8],

$$\Delta\mu_2^{0\#} = (\Delta\mu_1^{0\#}) + RT/ \tilde{V}_1^0 [B - (\tilde{V}_1^0 - \tilde{V}_2^0) \qquad (19)$$

where $\tilde{V}_0^2$ is the limiting partial molar volume of solute. The entropy of activation per mole of viscous flow of solution ($\Delta S_2^{0\#}$) at a particular temperature is determined by using the following relation,

$$\Delta S_2^{0\#} = -d\,(\Delta\mu_2^{0\#} / dT) \qquad (20)$$

Thus $\Delta S_2^{0\#}$ has been deduced from the slope of the plot of enthalpy of activation per mole of solute ($\Delta\mu_2^{0\#}$) against temperature. The enthalpy of activation, $\Delta H_2^{0\#}$ is evaluated from the following equation, [25]

$$\Delta H_2^{0\#} = \Delta\mu_2^{0\#} + T.\,\Delta S_2^{0\#} \qquad (21)$$

(Figure 1) represents the plot of variation of density (ρ) of aqueous solutions of pyridoxine and nicotinamide against molality (m) at T = 303.15 K. It is observed from the figure that density ρ of aqueous binary solutions of both systems increases linearly with concentration of solute. The same trend is observed at T/K = (298.15 and 308.15) for both vitamins. From the scrutiny of (tables 2 and 3), it is observed that the density of the βCD-aqueous binary mixture of both systems studied decreases slightly with rise in temperature from T/K = (298.15 to 308.15) at a particular concentration.

The variation of apparent molar volume of pyridoxine and nicotinamide (ϕ_V) in aqueous solution with concentration (m) at T/K = 298.15K - 303.15 is depicted in (figure 2). It is observed that ϕ_V^0 varies linearly with molality. The same type of behaviour is observed at all temperatures studied. From (Table 2), it is observed that ϕ_V increases with increase in temperature. (Figure 4) shows the variation of ($\phi_V - A_V\ .\ (C)^{1/2}$) against mass fractions of the solvent mixture at T/K = 298.15. A linear graph is obtained. Similar behaviour is observed at T/K = (298.15 and 308.15). From (table 2, 3), it is observed that ϕ_V^0 values for both vitamins increase with temperature. This may be due to the fact that at higher temperature, co-solvent (i.e., water + βCD) molecules are loosely held around the solute molecule. The same type of behavior is observed for nicotinamide by Banipal et al. [9] and Apelblat et al. [10] at higher temperatures. Kishore et al. [11] have also observed similar behavior for nicotinamide at T/K = (298.15 to 323.15). The values of ϕ_V^0 are found to be higher for pyridoxine than nicotinamide, which indicates that pyridoxine is more hydrated than nicotinamide when they are dissolved in water and has stronger solute-solvent interaction. The value of S_V, obtained from equation (3) and (7) generally represents volumetric and energetic effects of solute molecules [12, 13]. It can be observed from (table 2, 3) that the values of S_V are positive for both binary systems at all temperatures studied. The value of S_V suggests the presence of ion-ion interactions for electrolytes which is more in nicotinamide rather than pyridoxine [14, 15, 22]. It is seen that the value of S_V is more for nicotinamide than pyridoxine which indicates that (solute + solute)

interactions are stronger in nicotinamide as compared to pyridoxine when they are dissolved in (water+βCD) solution. [23] It is seen from (table 4) that the value of ($\delta^2\phi_V^0/\ \delta T^2$) for nicotinamide and pyridoxine at, (T = 298.15 K) is very small and positive. According to Hepler, the sign of ($\delta^2V^0/\ \delta T^2$) can be used for deciding the structure making and breaking tendencies of solutes when they are added in water [16]. For structure making solutes, the value of ($\delta^2V^0/\delta T^2$) is positive whereas it is negative for structure breaking solutes. Seen in this context, nicotinamide acts as a weak structure making and pyridoxine acts as strong structure maker when dissolved in co-solvent (water +βCD) at the temperature which has the maximum density. Pyridine has been reported as a structure breaker in aqueous solution [11]. This structure making ability of nicotinamide may be due to the $-CONH_2$ group present, conversely trend in values can be ascribed to the structural perturbation influenced by the gradual appearance of 'caging effect' or 'packing effect' [17, 18] in the studied solutions and it does not behave like common electrolytes and has hydrophobic character at higher temperatures. A more positive value of ($\delta^2\phi_V^0/\ \delta T^2$) suggests the predominance of hydrophobic hydration phenomenon over the electrostriction of (water+βCD) molecules around solute molecules. Thus, a higher value of ($\delta^2\phi_V^0/\ \delta T^2$) for the (hydroxyl+ primary alcoholic + methyl + (water+βCD)) indicates the presence of more hydrophobic hydration as compared to the (amide + water+βCD) system.

Cabani et al. [19] have shown that the coefficient of thermal expansion (α^*) can be used for understanding (solute + solvent) interactions. From (table 4), it is observed that the value of α^* at the elevated condition for aqueous solutions of pyridoxine is greater than those of nicotinamide. This is consistent with the results of the temperature derivative of ϕ_V^0 i.e. ϕ_E^0.

3.2. Transport properties

The variation of ($\eta_r - 1$) as a function of molarity (C) of solution for aqueous+βCD solutions of nicotininamide and pyridoxine at T/K = 298.15 is shown in (figure 5). It is observed that ($\eta_r - 1$) varies linearly with molarity for the above binary system. (Figure 5) shows a representative plot of ($(\eta_r - 1) / (C)^{1/2}$) against square root of molarity ($(C)^{1/2}$) for the aqueous+βCD solution of nicotinamide and pyridoxine at T/K = 303.15. It is observed from the above figure that ($(\eta_r - 1)/(C)^{1/2}$) varies linearly with $(C)^{1/2}$. A similar trend is observed for both aqueous binary systems of vitamins. From (tables 2 and 3), it is also seen that the viscosity (η_r) of aqueous

binary systems of pyridoxine and nicotinamide decreases with increase in temperature from T/K = (298.15 to 308.15) at a particular concentration.

The A-coefficient results from the electrostatic interactions between the ions. From (table 5), it is observed that the value of the A-coefficient is small and positive at all temperatures studied for (nicotinamide + water + βCD) & (pyridoxine + water + βCD), which means that there exist weak ion-ion interactions. The value of the A-coefficient is maximum at T/K = 298.15 and decreases on either side of temperature, which suggests that ion-ion interactions become weaker. Conversely in (pyridoxine + water +βCD), it is observed the values of A-coefficient is much small values which appears to be the weaker ion-ion interactions than nicotinamide system.

The second term in the Jones–Dole equation i.e. B coefficient is attributed to the influence on the hydrogen bond structure by the ion [19]. This provides information about the solvation of solutes, which reflects the (solute + solvent) interactions. It can be seen from (table 5) that viscosity B coefficient is positive for both binary systems at T = (298.15, 303.15 and 308.15) K suggesting the presence of strong (solute + solvent) interactions. The viscosity B coefficient for both systems, first decreases with increase in temperature and then increases with further rise in temperature. It is also observed that the B coefficient for the (pyridoxine + water) system is higher than (nicotinamide + water) indicating the presence of strong (solute + solvent) interactions.

The sign of the temperature derivative of B-coefficient (dB/dT) gives more important information regarding the structure making and structure breaking roles of solute in solvent media than the B coefficient. It is known that for structure making solutes, the dB/dT is negative and it is positive for structure breaking solute [20, 21]. Seen in this context, from (table 5) it is observed that value of dB/dT is minimum for T/K = 298.15 but it is maximum for T/K = 308.15 for both the systems studied. This suggests that in dilute (aqueous+ beta cyclodextrin) solutions, these solutes act as ‘‘structure makers’’ at lower temperature and ‘‘structure breakers’’ at relatively higher temperature.

The viscosity D coefficient explains (solute + solute) interactions for higher terms of coulombic forces. From (table 5), it can be seen that its value is positive for aqueous solutions of nicotinamide and pyridoxine at T/K = (298.15, 303.15 and 308.15), suggesting that (solute + solute) interactions are present at all temperatures. The number of water molecules surrounding a solute molecule is expressed in terms of the hydration number (η_H). From the

perusal of (table 5), it is seen that hydration number for nicotinamide decreases with increase in temperature, which means that extent of co-sphere (water+βCD) molecules decreases around each nicotinamide molecule with rise in temperature and vice versa in case of pyridoxine. The hydration number (η_H) for nicotinamide is found to be higher than that for pyridoxine at a particular temperature, which suggests that nicotinamide is more hydrated as compared to pyridoxine.

Eyring transition state theory has been used for interpreting viscous behavior of aqueous binary systems of studied vitamins. From (table 5), it is seen that the Gibbs free energy of activation per mole of solvent ($\Delta\mu_1^{0\#}$) values were found to be almost invariant of the solvent compositions at an experimental temperature but decrease slightly with a rise in temperature. However, ($\Delta\mu_2^{0\#}$) values are positive and much greater than for ($\Delta\mu_1^{0\#}$), all studied solutions at all the experimental temperatures suggesting the solute (ion)-solvent interactions in the ground state are stronger than in the transition state in case of vitamins. Hence in the transition state solvation of pyridoxine or nicotinamide is less favored in free energy terms.

According to Feakins et al. [19], the greater the value of $\Delta\mu_2^{0\#}$, greater is the structure-making tendency of a solute and the positive ($\Delta\mu_2^{0\#}$) values for pyridoxine in the studied solutions suggest it to be a net strong structure promoter/maker rather than nicotinamide.

It is known that entropy of activation per mole of viscous flow of solution ($\Delta S_2^{0\#}$) provides information about (solute + solvent) interactions. It is observed from (table 5) that for nicotinic acid, its value is large and positive at all temperatures studied which suggests that during viscous flow, the entropy of solution increases in going from the ground state to the transition state i.e. the activated state is less ordered as compared to the ground state. The value is found to be more negative for nicotinamide than pyridoxine, which implies that entropy of solution decreases (more in nicotinamide than pyridoxine) when the activated state is formed suggesting formation of a more ordered transition state. The enthalpy of activation per mole of solute, ($\Delta H_2^{0\#}$) which gives structural information of the solute species, has been evaluated by using equation (21), and its values are collected in (table 5). The values of ($\Delta H_2^{0\#}$) are found to be positive for vitamin B3 at temperatures studied suggesting that the transition state is strongly associated with H-bonding. The values of ($\Delta H_2^{0\#}$) along with ($\Delta S_2^{0\#}$)

are found to be negative for vitamin B6 at temperatures studied suggesting that the viscous flow to be exothermic and enthalpy driven.

Inspection illustrates that the values of (ϕ_v^o) increases with increasing mass fractions of β-CD and also found better for pyridoxine than nicotinamide, signifying the earlier interacts more. This may be explained as in case of pyridoxine, one extra hydrophobic group is encapsulated into the cavity of CD and the –OH groups of pyridoxine show higher ion-hydrophilic interaction with the –OH groups of CD. The larger diameter of β-CD helps in making more compact inclusion complex with the bioactive moiety, i.e., vitamin B6 than vitamin B3 relatively. Since the viscosity *B* values are again larger for β-CD–pyridoxine than β-CD–nicotinamide it suggests that inclusion is more constructive in case of former than the later. The structural feature of the vitamin B6 and CDs as explained earlier for which these trends of interactions have been acquired due to relative consequence of viscosity *B*-coefficient of the pyridoxine and CDs has been found analogous as for the case of densitometric studies.

3.3. Standard transfer volumes

Limiting thermodynamic transfer properties provide information about the solute-co-solute interaction, because at infinite dilution the interactions between individual solute molecules are negligible. Hence $\Delta_t\phi_v^0$ is free from solute-solute interactions and provides valuable information about solute-co-solute interactions. The standard partial molar volume of transfer ($\Delta_t\phi_v^0$) was obtained from the relation:

$$\Delta_t \phi_v^0 = \phi_v^0 \text{ [Vit } B_6 \text{ + aqueous–βCD] - } \phi_V^0 \text{ [water]} \qquad (22)$$

$$\Delta_t \phi_v^0 = \phi_v^0 \text{ [Vit } B_3 \text{ + aqueous–βCD] - } \phi_V^0 \text{ [water]} \qquad (23)$$

The ($\Delta_t\phi_V^0$) values are depicted in (Figure. 6) as a function of molality of Vit B_3 and Vit B_6 in two different comparable aqueous βCD solutions. According to the cosphere overlap model, as developed by Friedman and Krishnan [26], the overlap of hydration cospheres of two ionic species results in an increase in volume but that of hydration cospheres of hydrophobic-hydrophobic and ion-hydrophobic groups' results in net volume decrease. The positive ($\Delta_t\phi_v^0$) values indicate that ion-hydrophilic and hydrophilic-hydrophilic group interactions predominate over ion-hydrophobic, hydrophobic-hydrophobic and hydrophilic-hydrophobic interactions and viceversa for negative values.

The results are illustrated in (Table 6) and (Figure 6) as a function of molarity of aqueous β-CD solutions. Since the studied vitamins exist predominantly as in Vit B_3 there is the existence of (only hydrophilic amide group) and in Vit B_6 there is the presence of (methyl, two primary alcoholic groups which are hydrophobic in nature along with the hydrophilic hydroxyl group); therefore predominates in pure water and these leads to the overall decrease in volume of water due to electrostriction, the observed increasing positive volumes of transfer indicate that in the mixed solutions (Vit B_6 + aqueous. β-CD), have the ion–hydrophobic and hydrophilic–hydrophilic group interactions predominate over the ion–hydrophobic and hydrophobic–hydrophobic groups interactions, and the contribution increases with the molality of β-CD in solutions. But in case of mixed solutions of (Vit B_3 + aqueous βCD) though it behaves in a similar manner like the aforesaid system but here the hydrophilic–hydrophilic group interactions outweigh the ion-hydrophobic interactions due to the absence of hydrophobic side chains.

($\Delta_t\phi_V^0$) values are positive at all the experimental temperatures and increases monotonically with the increase in vitamins content in the mixed solutions.

The partial molar volume of a solute can also be explained by a simple model [27, 28] as given by the relation:

$$\phi_V^0 = \phi_{VW} + \phi_{Void} - \phi_S \qquad (24)$$

Where (ϕ_{VW}) is the Van der Waals volume, (ϕ_{Void}) is the volume associated with voids or empty space, and ϕ_S the shrinkage volume due to electrostriction. Assuming the (ϕ_{VW}) and (ϕ_{Void}) to have same magnitudes in water and in aqueous β-CD solutions for the same solutes (i.e., Vit B_3 and Vit B_6), the increase in ϕ_V^0 values and the concomitant positive ($\Delta_t\phi_V^0$) values can be attributed to the decrease in the shrinkage volume (ϕ_S) of water by pyridoxine and nicotinamide in presence of β-cyclodextrin. This fact suggests that β-CD has almost certainly a dehydration effect (i.e., due to the presence of inner hydrophobic core in its structure) on the hydrated Vit B_6 and Vit B_3. Thus the interactions between pyridoxine and β-CD can roughly be summarized as follows: (i) interaction of the pyridine–N-group of pyridoxine with H^+, OH^- ions of water in a minimal amount.

(ii) (a) Interaction of the –H–O–H– at the wider rim of β-cyclodextrin, between –OH group of pyridoxine with the –OH group present in the wider cavity of the βCD. (b) Interaction between the $–CH_2OH$ group of pyridoxine with –OH group of cyclodextrin again in the wider

rim of the truncated bucket of cyclodextrin are firm enough (i.e., the hydrophilic – hydrophilic interaction) which are the driving forces to thrust the hydrophobic moiety inside the cavity of the βCD and (iii) ionic–hydrophobic interaction between the –H– ion of the cyclodextrin i.e., (H3 and H5 hydrogen's as detected from the NMR Spectroscopy, discussed later in these work) with the ($–CH_3$ group of the pyridoxine and pyridine ring of the pyridoxine), i.e., the non-polar part of pyridoxine molecules which is an assorted interaction.

Interactions between nicotinamide and β-CD can roughly be summarized as follows: (i) interaction of the pyridine– N-groups of with H^+, OH^- ions of water in a minimal amount. (ii) (a) Interaction of the –C–O–H– at the wider rim of β-cyclodextrin, between –CO– group of Vit B_3 with the –OH group present in the wider cavity of the βCD. (b) Interaction between the $–NH_2$ group of Vit B_3 with –OH group of cyclodextrin again in the wider rim of the truncated bucket of cyclodextrin are firm enough (i.e., the hydrophilic – hydrophilic interaction) are much predominant here and (iii) moreover the hydrophobic interaction in the inner part of the cavity between the cyclic pyridine ring of the nicotinamide with the (H3 proton detected from NMR spectra) is another assorted interaction.

While interactions of (i)-(ii): (a), (b) types impart positive contributions, interaction of (iii) types imparts negative contribution to Φ_v^0 values in both the studied system thereby suggesting that the irregular trend in ($\Delta_t\phi_V^0$) values indicating the presence of complex interactions between solute and co-solute in aqueous βCD solutions (containing background 0.005 M of the solvent mixture). Therefore, the overall positive Φ_v^0 values indicate that ionic group interactions outweigh over ionic-hydrophobic interactions. Nonetheless, standard partial molar volumes of a solute reflect a overall result of several solute-solute and solute-solvent interactions prevailing in solutions such as: Electrostatic interactions connecting the local charge on the solute or ions and the dipole moment of H_2O, interlocking packing interactions of the solute or ions with H_2O leading to interstitial packing or caging over and above solvation, and other polar-ionic group (H-bonding) interactions between different polar and non-polar groups of β-cyclodextrin and vitamins studied; all these interactions can characterize the overall state of the solutions studied.

3.4 UV-Vis Spectroscopic study

The absorption spectrum of aqueous β-cyclodextrin (1×10^{-4} mol. L^{-1}) solution did not have any considerable absorption band in the wave length range (200–400) nm, hence neglected. The absorption spectra of IC of (Vit B3 + βCD) and (Vit B6 + βCD) in aqueous solutions at (1×10^{-4} mol. L^{-1}) concentration content at 298.15 K are shown in (Figure. 8, 9) accordingly. For IC of Vit B6, it is observed that as we increase the concentration of Vit B6 the intensity of UV absorption decreases in a continuous fashion but in case of IC of Vit B3 the decrement of absorbance was not well observed. So, we can say IC happening better in case of Vit B6, rather than Vit B3. This shift in absorption stands in support of the diverse possible interactions Vit B6 with aqueous β-CD solutions i.e. solute-solvent interactions as already discussed.[29-31]

3.5 Fluorescence Study

In case of fluorescence data for Vit B6 in IC at various concentrations, the fluorescence emission was observed at 380 nm with a noticeable intensity decrement whereas in case of Vit B3 the fluorescence intensity decrement was not profound; fluorescence emission in was observed at 405 nm. So, we can conclude that Vit B6 serves a better job forming a prominent IC over Vit B3.[31-33]

3.6 Infrared study

In the case of nicotinamide, the In plane and Out plane vibrations shows that the substitution C=O and $–NH_2$ do not affect much of aromatic C–H modes of vibration as they attached to only one carbon of the benzene ring at α position. The deviation of C=C stretching mode may be due to the presence of very strong (N–H) in-plane bending modes which also lie closer to that range of frequencies. Because of the title molecule not purely benzene (hetero aromatic), the position of C–C stretching band is slightly dislocated. The strong intensity of the band may be due to the mixing of C= N with C=C, both occurring at the same frequency. The C–N stretching vibrations are always mixed with other bands and normally occur in the region 1266–1382cm^{-1}. Another band observed at 1154 cm^{-1} which may be due to the C–N present in amide group. This shows a considerable difference between vibrations of C–N bonds, when they are present within the ring and outside the ring. The title molecule is a type of amino pyridine which is comprises of carbonyl and amino groups. N-H bands show a slight deviation from the expected range which may be due to the interaction of C=C stretching

vibrations. Frequencies slightly differ with the literature values in C-NH_2. C=O vibration not influenced by the other substitution in the chain. But when the inclusion complex is formed then it is found that the stretching and bending vibrations of the C=O, -NH_2, C-O-C, C=O, C=N are shifted and in some cases merged which proves the fact that the pyridine ring is inserted in the cavity of the cyclodextrin.[37]

On the other hand in case of pyridoxine all the major band assignments are represented in (Table 7) are overviewed in the study. When the pyridoxine is complexes with β-CD then the position of the vibrations are shifted or changed more in comparable to that of nicotinamide, which again proves the fact the inclusion with pyridoxine, is best suited.[34-36]

3.7 NMR Spectroscopy

Insertion of a guest molecule into the hydrophobic cavity of cyclodextrin results in the chemical shift of the guest as well as of the cyclodextrin molecule in NMR spectra, which is due to the interaction of the host with the guest molecule. In the case of aromatic Bio-active compounds, the spectral changes that can be observed upon inclusion are the diamagnetic shielding of the aromatic guests with the interacting atoms of the host molecule. In the structure of cyclodextrin, the H3 and H5 hydrogen's are situated inside the conical cavity; in particular, the H3 are placed near the wider rim, whereas H5 are placed near the narrower rim of the cyclodextrin molecule.

The other, H1, H2 and H4, hydrogens are located at the exterior of the cyclodextrin molecule (Scheme). The molecular interactions have been studied by 1H NMR spectra. Upon inclusion the signals of H3 and H5 of cyclodextrin as well as the interacting aromatic protons show considerable upfield shift. In this study, the guest vitamin B6 also contains the two hydrophilic –CH_2 (OH) – and -OH groups and signals of which merge with that of the H3 and H5 of cyclodextrin. Therefore, it is better to investigate the chemical shifts of the aromatic protons here rather than that of the H3 and H5 of cyclodextrin (though upfield chemical shift is observable for H3 and H5, the values are not clear), (Figure 13, 14). The shifts of vitamin B3 on the other hand when they interact with β-CD are negligible.

Therefore, it is clear that vitamin B3 do not form better inclusion complexes with β-CD, whereas vitamin B6 form inclusion complexes with β-CD. In the case of β-CD, considerable chemical shifts are observed for the two vitamins, confirming the formation of inclusion complexes between the two different vitamins with β-CD (Figure13, 14).[38, 39]

3.8 SEM Technique

Electron microscopy (SEM) is a very renowned technique for analyzing the surface texture and particle size of solid materials. The surface morphological structures of β-CD and the solid IC (vit B3: β-CD, vit B6: β-CD) are shown in (Figure. 15, 16) respectively. From (Figure 15, 16) it is clear that the morphological structures that they are totally different from each other. Moreover as the complexation by β-CD can be viewed distinctly. This gives clear evidence that [vit B6] fits enough into the hydrophobic cavity of β-CD to form of solid IC.

3.9 HRTEM Technique

TEM is a consistent method for studying aggregation was employed to investigate the complexation of the oligosaccharides and the vitamins formed in aqueous solution. The morphology of the obtained supramolecular assembly is reflected in TEM images (Figure. 17, 18).

3.10. Powder X-ray Diffraction Pattern

The direct evidence for the complexation between host and guest molecule can be obtained from the analysis of powder X-ray diffraction spectrum. [Figure. 19, 20] shows that the diffraction pattern and the characteristic peak intensity of the resultant IC are quite different from the diffractogram of Vit B3 monomer which suggests the formation of a new phase of solid inclusion complex. The characteristic peaks of Vit B3 are assigned at 2θ (degree) values of 1.08, 11.87, 15.40, 19.68, 20.12, 20.49, 22.81, 23.32, 23.95, 25.29, 26.42, 27.92, 29.01, 30.72 (Figure. 19a) and these peaks were not overlay with the peaks of the solid inclusion complex (Figure. 19b). In β-CD: Vit B3 solid, the new peaks appeared at 2θ (degree) values of 3.27, 15.45, 20.19, 21.51, 23.98, 26.57, 28.18, 33.47, 37.90, 42.02, 53.14.

In the next case the characteristic peaks of Vit B6 are assigned at 2θ (degree) values of 10.07, 14.91, 16.24, 19.42, 19.70, 20.64, 21.89, 23.07, 24.49, 25.28, 25.66, 26.96, 27.67,

28.40, 30.12 (Figure 20a) and these peaks were not overlay with the peaks of the solid inclusion complex (Figure 20b). In β-CD: Vit B6 solid, the new peaks appeared at 2θ (degree) values of 1.13, 10.07, 11.33, 12.38, 13.18, 15.03, 16.13, 17.90, 20.15, 20.84, 23.54, 24.62, 25.48, 27.01, 27.74. This study clearly established that Vit B6 forms IC successfully with β-CD rather than the IC of Vit B3.

3.11 Acute Toxicity Test

In the acute toxicity test, three rat groupsconsisting of mature rats were selected, each containing 6 rats (3 males and 3 females). The first group was considered as normal (N) which was not fed with *Aloe vera* gel. The other two were experimental groups, designated as TA1 and TA2. TA1 animals were fed with 2 g *Aloe* crude gel/kg b. w. and TA2 were fed with *Aloe* crude gel at the rate of 5g/kg b. w. The animals were fasted overnight prior to feeding with a single dose of the gel homogenate. The mortality rate, salivation, fur irritation, sleep/dizziness, lethargy, and diarrhea were observed for the next 24 hours. This one-day long observation was further followed by a 7-day long screening for any long-term toxicological effects. All the experiments were done according to the OECD guideline 423 (Adopted 17th December 2001) for the acute oral toxicity study in rodents.

3.12 Sub-chronic Toxicity

For the determination of sub-chronic toxicity, animals were divided into four groups. All the groups contained 6 rats each (3 male and 3 females). The first group was considered as Normal (N), the second, third and fourth groups were treatment groups with a 28 day long daily feeding schedule of the two inclusion complexes (designated as T1, T2, and T3 respectively). After the completion of 28 day-long schedule, animals were sacrificed through anesthesia; blood from each animal was collected separately by puncturing their hearts with sterile needles. Kidney and livers tissues were collected by separating out the organ from the body and were fixed in 4% formalin for histological processing. Blood was collected in EDTA vials to assess the complete blood profile. The clear serum was collected by allowing the blood samples to clot. Hemoglobin was measured immediately after the blood collection. All the experiments were done according to the standard guidelines with slight modifications[11].

In vitro anti-inflammatory activity:

Membrane stabilizing activity

Hypotonic solution-induced hemolysis

The effect of the AUME on hRBC hemolysis induced by hypotonic solution was assessed by a modified method of Shinde et al., (1999). For the test, AUME was dissolved at a concentration of 100, 200 and 400 µg/ml in 5 ml of hypotonic solution (distilled water) in one set of centrifuge tubes. In another set of centrifuge tubes, isotonic buffer solution (5 ml) containing similar graded doses of the AUME (100, 200 and 400 µg/ml) were taken. In standard group, Indomethacin (200 µg/ml) was used as a standard drug. Control tubes contained only 5 ml of the distilled water as hypotonic solution and no AUME or standard drug was added. The experiments were carried out in triplicate pairs (per dose) of the centrifuge tubes.100µl of stock erythrocyte suspension was then added to each of the centrifuge tubes and after gentle mixing, the mixtures were incubated for 1 hour at 37°C temperature. After incubation, the mixture was centrifuged for 3 min at 1300 g in room temperature and the absorbance (OD) of the supernatant was measured at 540 nm using double beam UV- VIS spectrophotometer (Ray-Leigh UV-2601, Beijing, China). The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water (hypotonic solution) to be 100%. Thus the percent inhibition of haemolysis was calculated using the following equation.

% Inhibition of haemolysis = $[1-(OD_2-OD_1)/(OD_3-OD_1)] \times 100$

Where,

OD_1 = Absorbance of test sample in isotonic solution

OD_2 = Absorbance of test sample in hypotonic solution

OD_3 = Absorbance of control sample in hypotonic solution

Heat induced hemolysis

This test was carried out with the standard protocol describes by Shinde et al., (1999) with minor modifications. For this test, one untreated group, three experimental AUME treated groups and one standard drug group were considered. Centrifuged tubes were arranged in

triplicate sets (3 sets per dose). First set, designated as control tubes contained only 5 ml of the distilled water (vehicle). In the experimental groups, AUME were dissolved in 5ml of isotonic phosphate buffer solution (pH 7.4) at a concentration of100, 200 and 400µg/ml. The standard drug dose group contained 5ml isotonic buffer solution containing indomethacin at a concentration of 200 µg/ml. An amount of 100µl of erythrocyte suspension was added to each tube and mixed gently. The tubes were incubated at 54°C for 20 min in a regulated water bath. After incubation period, the reaction mixture was centrifuged at 1300 g for 3 min in room temperature and the absorbance (OD) of the supernatant was measured at 540 nm. The percent inhibition of hemolysis by the AUME was calculated using the following equation (Tatiya and Saluja, 2011):

% Inhibition of haemolysis = $(OD_2 – OD_1)/OD_2 \times 100$

Where,

OD_1 = Absorbance of heated test sample

OD_2 = Absorbance of heated control sample

Protein denaturation test

The test was performed as described by the published protocols (Mizushima and Kobayashi, 1968; Gupta et al., 2015) with minor modifications. Egg albumin was used as protein source and the protein concentration was measured by commercial Folin-Lowry method-based biochemical kit following the manufacturers' protocol. The experiment was carried out in triplicate pairs (per dose). The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of AUME resulting in the final reaction concentrations of 100, 200, 400µg/ml. 2ml of distilled water served as the control group instead of AUME. Diclofenac sodium (100µg/ml) was used as a standard drug. The reaction mixture was incubated at 37°C for 15 minutes in incubator and then the mixture was heated at 70° C for 15 minutes in a regulated water bath. The resulting solution was cooled down to room temperature and the turbidity of the solution was measured spectrophotometrically at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula:

% Inhibition of protein denaturation = $(OD_2 – OD_1)/OD_2 \times 100$

Where,

OD_1 = Absorbance of heated test sample

OD_2 = Absorbance of heated control sample

Statistical analysis

All statistical analyses were done using GraphPad prism version 6.01. Data were reported as mean ± S.E.M. (Standard Error Mean) and were analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test. The results were considered statistically significant at $P \leq 0.05$compared to the control group (distilled water). The **** denotes significance value at $P < 0.0001$.

Conclusion

The present study explains that nicotinamide and pyridoxine form ICs with *β*-CD in aqueous medium, which can be used as regulatory releaser of the above two vitamins. NMR study confirms the inclusion phenomenon and its mechanism. The thermodynamic parameters have been estimated for both the ICs by reliable spectroscopic techniques with high accuracy, which inform that pyridoxine-*β*-CD has higher order of complexation than that of nicotinamide-*β*-CD. Thus, this work communicates both qualitative and quantitative idea about the formation of ICs of *β*-CD with above two vitamins suggesting their potential applications in pharmaceutical industries in controlled manner and medical sciences, proved by acute and sub –chronic toxicity along with In-Vitro analysis.

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TABLE 1

Provenance and mass fraction purity of chemical samples

Chemical name	Source	Analysis method	% Water content (w/w)	Mass fraction purity (w/w)
Nicotinamide	Sigma Aldrich	LC–MS[a]	0.05[b]	0.999
Pyridoxine	Sigma Aldrich	LC–MS[a]	0.04[b]	0.999

TABLE 2

Density (ρ), [a] Limiting apparent molar volume of solute (ϕ_V^0), experimental slopes (S_V), absolute viscosity (η) and relative viscosity(η_r) of an aqueous solutions of pyridoxine at different temperatures T = (298.15, 303.15 and 308.15) K at 10^5 N. m^{-2}.

T/K	Co-solvent mixture in mass fractions(w)	10^{-3} ρ/kg. m^{-3}	10^6 ϕ_V^0/m^3. mol^{-1}	$S_V.10^6/(m^3.mol^{-2}.kg)$	10^3 η/Pa. s	η_r
298.15	0.001[a]	0.99751	145.31 (±1.03)	27.86 (±0.09)	0.99689	0.76683
		0.99786	145.25 (±1.03)	27.66 (±0.09)	0.99756	0.76735
303.15		0.99628	146.49 (±1.04)	26.67(±0.08)	0.88673	0.73894
		0.99672	146.56 (±1.04)	26.42(±0.08)	0.89324	0.74436
308.15		0.99507	145.70 (± 1.03)	25.68 (±0.12)	0.77651	0.70591
		0.99558	145.93 (± 1.04)	25.41(±0.12)	0.78892	0.71720
298.15	0.003[a]	0.99777	146.19 (±1.03)	24.95 (±0.11)	0.99703	0.76694
		0.99792	146.36(±1.03)	24.63 (±0.11)	0.99768	0.76744
303.15		0.99674	145.69 (± 1.04)	23.98 (±0.09)	0.89653	0.74710
		0.99699	145.70 (±1.04)	23.77(±0.09)	0.80342	0.75285
308.15		0.99552	146.95(±1.02)	23.65(±0.10)	0.78441	0.71310
		0.99576	146.75 (±1.02)	22.89(±0.10)	0.79008	0.71825
298.15	0.005[a]	0.99801	146.19 (±1.05)	22.65 (±0.12)	0.99843	0.76802
		0.99809	146.15 (±1.05)	22.62 (±0.12)	0.99788	0.76760
303.15		0.99674	147.42 (±0.98)	22.60(±0.10)	0.90661	0.75550
		0.99609	147.22 (±0.98)	22.59(±0.10)	0.91246	0.76038
308.15		0.99574	146.80 (±0.99)	22.59 (±0.14)	0.79006	0.71823
		0.99663	146.68 (±0.99)	22.56(±0.14)	0.80886	0.73532

[a] Standard uncertainties u are u (T) = 0.002 K; u(m) = 0.0001 mol . kg^{-1}, u(p) = 7.0 . 10^2 N . m^{-2} and combined expanded uncertainty U*p*(*p*) = 0.1 kg . m^{-3} with 0.95 level of confidence (k = 2).

TABLE 3

Density (ρ), [a] Limiting apparent molar volume of solute (ϕ_V^0), experimental slopes (S_V), absolute viscosity (η) and relative viscosity(η_r) of an aqueous solutions of nicotinamide at different temperatures T = (298.15, 303.15 and 308.15) K at 10^5 N. m^{-2}.

T/K	Co-solvent mixture in mass fractions	10^{-3} ρ/kg. m^{-3}	10^6 ϕ_V^0/m^3. mol^{-1}	$S_V.10^6/(m^3.mol^{-2}.kg)$	10^3 η/Pa. s	η_r
298.15	0.001[a]	1.00195	93.08	124.28	1.47200	1.03011
		1.00278	93.18	124.25	1.48053	1.03363
303.15		1.00244	94.06	121.62	1.32981	1.02599
		1.00278	93.85	121.51	1.32887	1.02794
308.15		1.00257	94.26	119.78	1.23411	1.01543
		1.00293	94.59	119.87	1.26412	1.01931
298.15	0.003[a]	1.00208	94.17	124.22	1.48229	1.03038
		1.00296	93.27	124.32	1.48496	1.03294
303.15		1.00256	94.17	121.11	1.32776	1.02188
		1.00289	93.93	121.38	1.32756	1.02195

308.15		1.00268	94.42	119.68	1.24865	1.01025
		1.00308	94.67	119.82	1.25561	1.01493
298.15		1.00211	94.25	124.12	1.48498	1.03000
		1.00315	94.38	123.87	1.48708	1.03269
303.15	0.005[a]	1.00267	94.31	121.42	1.32678	1.02272
		1.00297	93.97	121.20	1.32632	1.02432
308.15		1.00277	94.61	119.52	1.26788	1.01964
		1.00319	94.83	119.32	1.24221	1.01802

[a] Standard uncertainties u are u(T) = 0.002 K; u(m) = 0.0001 mol . kg $^{-1}$, u(p) = 7.0 . 10^2 N . m $^{-2}$ and combined expanded uncertainty $U_p(p)$ = 0.1 kg . m $^{-3}$ with 0.95 level of confidence (k = 2).

TABLE 4

Standard deviation (u) in (ϕ_V^0), absolute average deviation (AAD), in (ϕ_V^0), limiting apparent molal expansivity of solute (E^0_ϕ) at T/K = (298.15 to 308.15), $\delta^2\phi_V^0/\delta T^2$ and coefficient of thermal expansion (α^*) at T = 298.15 K of both aqueous β-CD binary systems with mass fraction (w=0.005).

T/K	$10^3\sigma$	AAD	106 $E\phi^0/m^3$. mol^{-1} . K^{-1}	$\delta^2\phi_V^0/\delta T^2$	$10^3\alpha^*/K^{-1}$	$\Delta_t\phi_V^0$
Nicotinamide + water + βCD						
298.15	0.2041	0.0967	23.029	0.0225	1.65	0.72
303.15	0.2397	1.1627	21.987			0.77
308.15	0.2685					0.69
Pyridoxine + water + βCD						
298.15	0.1321	1.0086	32.107	0.4682	2.84	0.79
303.15	0.1O02	1.0094	29.762			0.82
308.15	0.1631	1.0132				0.66

TABLE 5

Experimental values of Falkenhagen coefficient A, Jones-Dole coefficient B and D viscosity coefficients, temperature coefficient of B, i.e. (dB/dT), Gibbs free energies of activation per mole of solvent ($\Delta\mu_1^{0\#}$) and per mole of solute ($\Delta\mu_2^{0\#}$), entropy of activation per mole of solute ($\Delta S_2^{0\#}$), enthalpy of activation per mole of solute ($\Delta H_2^{0\#}$) at and hydration number (n_H), for both aqueous binary systems at T/K = (298.15, 303.15 and 308.15), (w = 0.005).

T/K	A (dm^3 . mol^{-1}) $^{1/2}$	B (dm^3 . mol^{-1})	D (dm^3.mol^{-1})^2	dB/dT	($\Delta\mu_1^{0\#}$)kJ .mol^{-1})	$\Delta\mu_2^{0\#}$(kJ .mol^{-1})	$\Delta H_2^{0\#}$ (kJ . mol^{-1})	T.$\Delta S_2^{0\#}$ (kJ . mol^{-1})	n_H
Nicotinamide + water +βCD									
298.15	0.2275 (±0.001)	0.2713 (±0.011)	0.7820	-0.0329	9.79 (±0.01)	46.42 (±0.10)	42.75	-3.98	4.93
303.15	0.0056 (±0.001)	0.2841 (±0.014)		0.6789	9.45 (±0.01)	55.58 (±0.10)	54.03	-3.99	3.97
308.15	0.0015 (±0.001)	0.2586 (±0.016)		0.8521	9.32 (±0.01)	56.11 (±0.10)	54.08	-4.00	3.87
Pyridoxine + water + βCD									
298.15	0.011 (±0.001)	0.381 (±0.016)		-0.0598	9.89 (±0.01)	82.05 (±0.10)	-321.56	-290.86	2.78

303.15	-0.08 (±0.001)	0.425 (±0.017)		0.1345	8.81 (±0.01)	92.73 (±0.10)	-237.81	-291.78	2.65
308.15	-0.012 (±0.001)	0.462 (±0.009)		0.4382	8.73 (±0.01)	103.56 (±0.10)	-189.00	-291.91	2.32

Table 6

Estimated vibrational frequencies for [β – CD: nicotinamide] Complex formation

Nicotinamide	
wave number / cm^{-1}	Group
3100–3000cm^{-1} 3060 cm^{-1} (assigned to C-H stretching vibration)	Hetero aromatic organic compounds and its derivatives are structurally very close to benzene and commonly exhibit multiple weak bands in the region. Due to C–H stretching vibrations.
1300–1000cm^{-1} The bands for C–H in-plane bending vibrations of the title compound are identified at 1126, 1093 and 1030cm^{-1}.	C–H in-plane ring bending vibrations normally occur as a number of strong to weak intensity sharp bands in this region.
900–667cm^{-1} In the present case, the bands are identified at 963 and 829 cm^{-1} in for C–H out-of-plane bending.	The C–H out-of-plane bending vibrations are strongly coupled vibrations and occur in the region
1400 and 1650cm^{-1} C=C stretching vibrations have been found at 1404, 1486 cm^{-1}.	The (C=C) stretching modes are normally observed between in benzene derivatives.
1423 cm^{-1}	The C–C stretching peaks are also found
In the present work, two strong bands present at 626 and 605 cm^{-1} assigned to CCC in-plane bending.	The CCC bending bands always occur below 600cm^{-1}
The present work shows the presence of C=N stretching vibration at 1593 cm^{-1} for with strong intensity.	The pyrimidine and its related compounds show a strong absorption band in the region 1600 -1500cm^{-1} due to C= N ring stretching vibration.
1154 cm^{-1}	Band observed may be due to the C–N present in amide group.
The band is assigned at 3367cm^{-1}	In pyridine molecule, the N–H stretching vibration usually observed in the region 3000–3500cm^{-1}.
1621–1593cm^{-1}	The N–H in-plane bending vibrations usually occur in the region.
Assigned at 779 and 703cm^{-1}	N–H out-of-plane, bending vibrations
1126 cm^{-1}	C–NH_2 stretching vibration is observed
1699 cm^{-1}	C= O stretching vibration
513cm^{-1}	C= O out-of-plane, bending vibration
β-CD	
wave number / cm-1	Group
3349.23cm^{-1}	stretching of O-H
2919.12cm^{-1}	stretching of –C-H from –CH2
1409.18cm^{-1}	bending of –C-H from –CH2 and bending of O-H
1153.17cm^{-1}	bending of C-O-C
1033.02cm^{-1}	stretching of C-C-O
938.64cm^{-1}	skeletal vibration involving α-1,4linkage
β-CD +[Nicotinamide]	
wave number / cm-1	Group
–	Streching of O-H is absent

–	Stretching of –C-H from –CH2 absent.
1524cm-1	Stretching of $-NH_2$ ion of nicotinamide
1406cm^{-1}	bending of –C-H from –CH2 and bending of O-H
1162cm^{-1}	bending of C-O-C of β-CD
1046cm^{-1}	stretching of C-C-O of β-CD
610cm^{-1}	C=O out of plane stretching in nicotinamide shows deviation.
568cm^{-1}	C=O out of plane bending vibration is also shifted.

Table 7

Estimated vibrational frequencies for [β – CD: pyridoxine] Complex formation

Pyridoxine	
wave number / cm-1	Group
3340cm^{-1}	Stretching of phenolic –OH
3250cm^{-1}	Stretching of alcoholic -OH
1630 cm^{-1} and 1550 cm^{-1}	C=C and C=N aromatic ring
1278cm^{-1}	C=O stretching of the aryl alkyl group ether
1225-1200cm^{-1}	C-O stretch in vinyl ether
1070 cm^{-1}	C-O stretch of primary alcohol
1570cm^{-1}	N-H bending in amine
β-CD	
wave number / cm-1	Group
3349.23cm^{-1}	stretching of O-H
2919.12cm^{-1}	stretching of –C-H from –CH2
1409.18cm^{-1}	bending of –C-H from –CH2 and bending of O-H
1153.17cm^{-1}	bending of C-O-C
1033.02cm^{-1}	stretching of C-C-O
938.64cm^{-1}	skeletal vibration involving α-1,4linkage
β-CD +[Pyridoxine]	
wave number / cm-1	Group
3324.68cm^{-1}	Stretching of O-H has shifted.
2840.56cm^{-1}	Symmetrical stretching of–C-H from –(CH_3) and –CH_2 of pyridoxine
1463.23cm^{-1}	C=N stretching of the aromatic ring is shifted.
1020.78cm^{-1}	C-O stretching of the primary alcohol is shifted.
997cm^{-1}	C-H bending at 1, 3-disubstituted region at the opening of the wider cavity of the cyclodextrin by pyridoxine.
760cm-1	C-H bending at 1,2 disubstituted and mono substituted benzene derivative of pyridoxine.

FIGURES

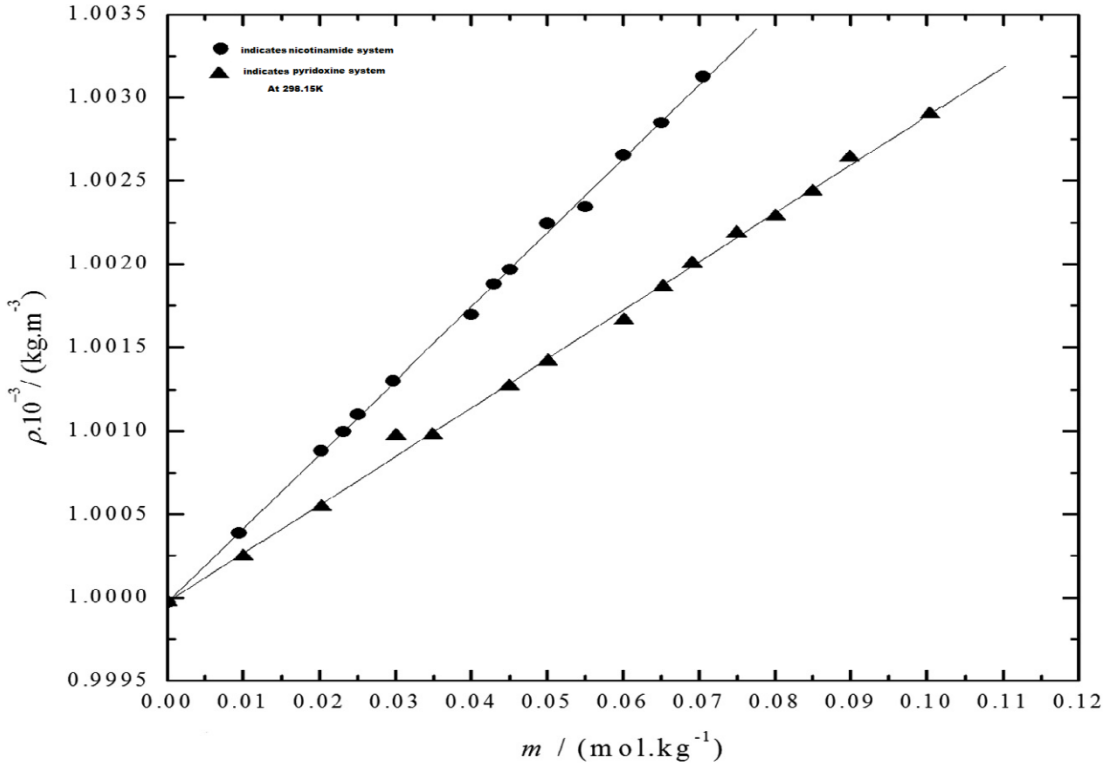

Figure1. Plot of the variation of density (ρ) of solution as a function of molality (m) for aqueous solutions of pyridoxine at T = 298.15 K: Δ-Δ, nicotinamide at T = 298.15 K: O-O.

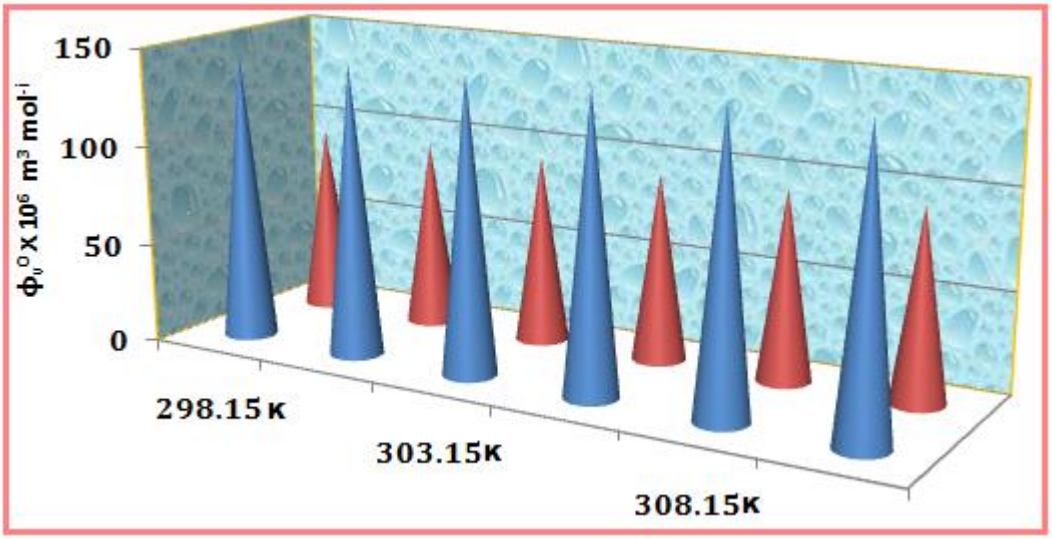

Figure 2. Dependence of apparent molar volumes (ϕ^0_V) in 0.005 mass fraction of pyridoxine (blue bars) & nicotinamide (brown bars) in aqueous β CD solutions at (T =298.15–308.15) K.

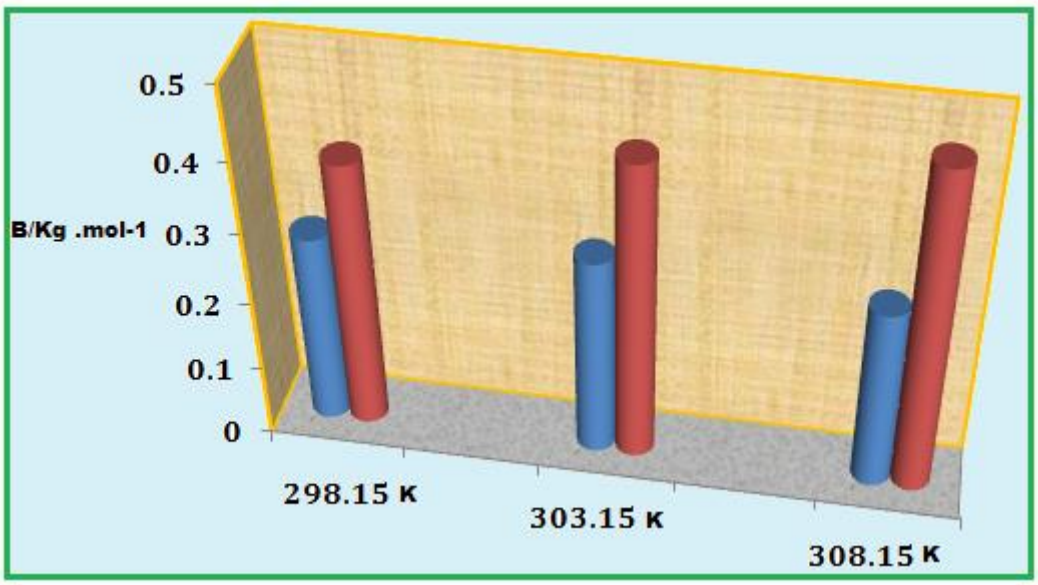

Figure3. Plots of viscosity B-coefficients for pyridoxine (brown bars) and nicotinamide (blue bars) in mole fraction (0.005) in aqueous solutions at T = (298.15–308.15) K.

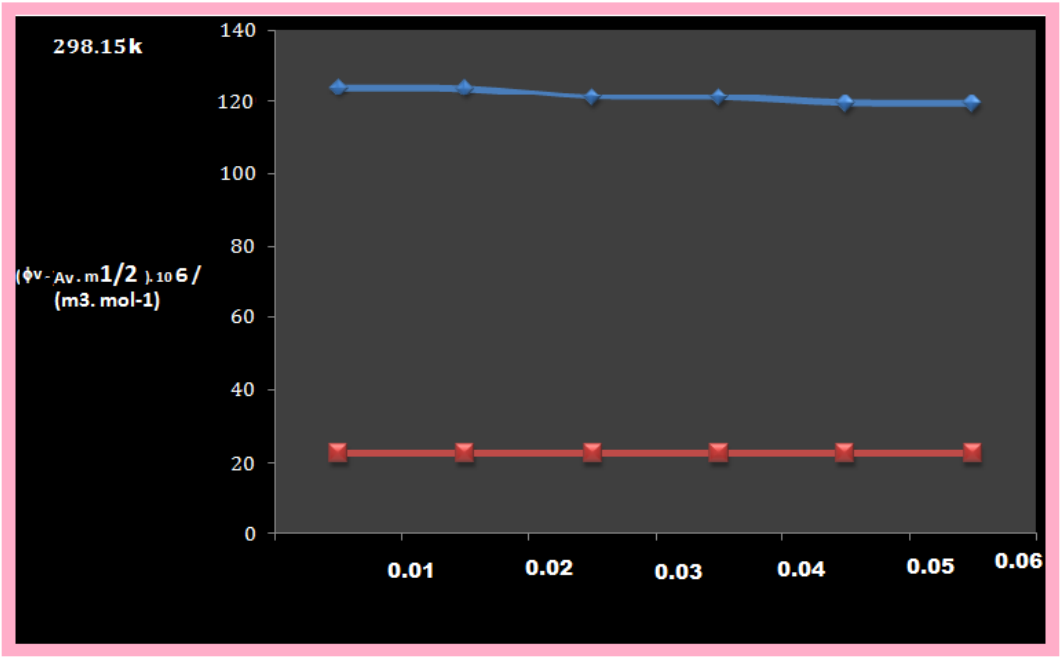

Figure4. Plot of the variation of (ϕ_V - A_V (m) $^{1/2}$) as a function of various intermediate mass fractions of (0.005) in terms of molality/mol. Kg^{-1} for aqueous βCD solutions of nicotinamide (blue colored linear graph) & pyridoxine (brown colored linear graph) at T = 298.15 K to 308.15K.

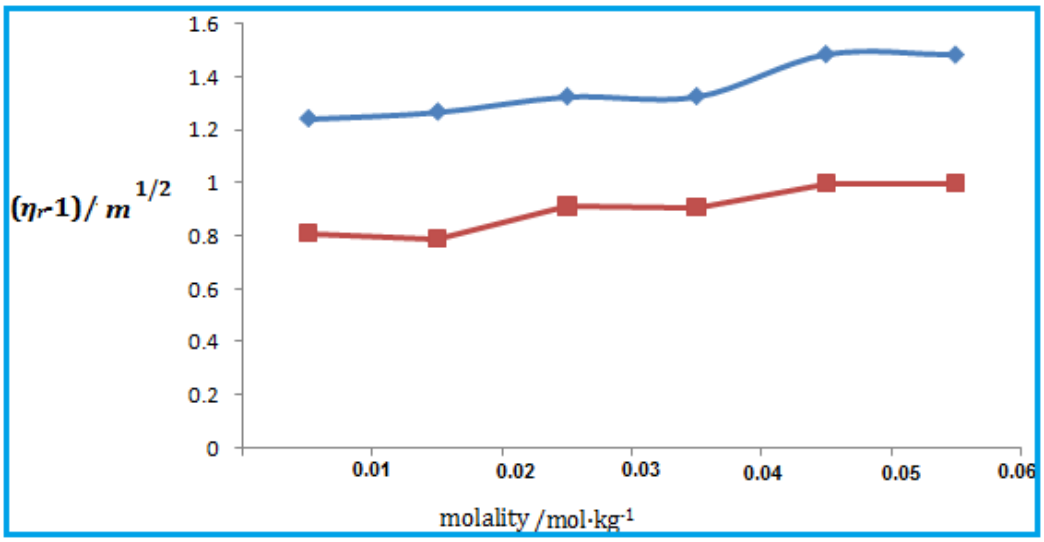

Figure5. Plot of the variation of $((\eta_r\text{-}1) / \sqrt{m^{1/2}})$ as a function of $(C^{1/2})$ for aqueous βCD solutions of nicotinamide (denoted with brown line) & pyridoxine (denoted with blue line) at the elevated temperature of T = 298.15 K.

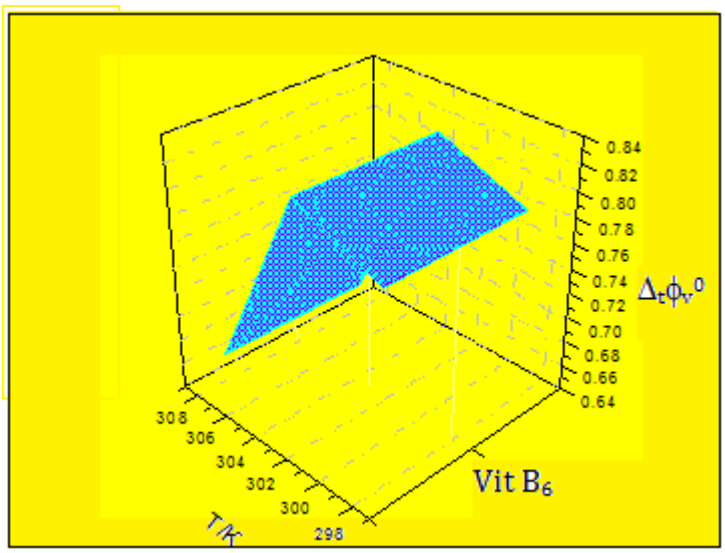

Figure6. Plots of standard partial molar volume of transfer ($\Delta_t\phi^0_V$) for pyridoxine on the molality of beta-cyclodextrin (m βCD) in aqueous solutions at T = (298.15–308.15) K.

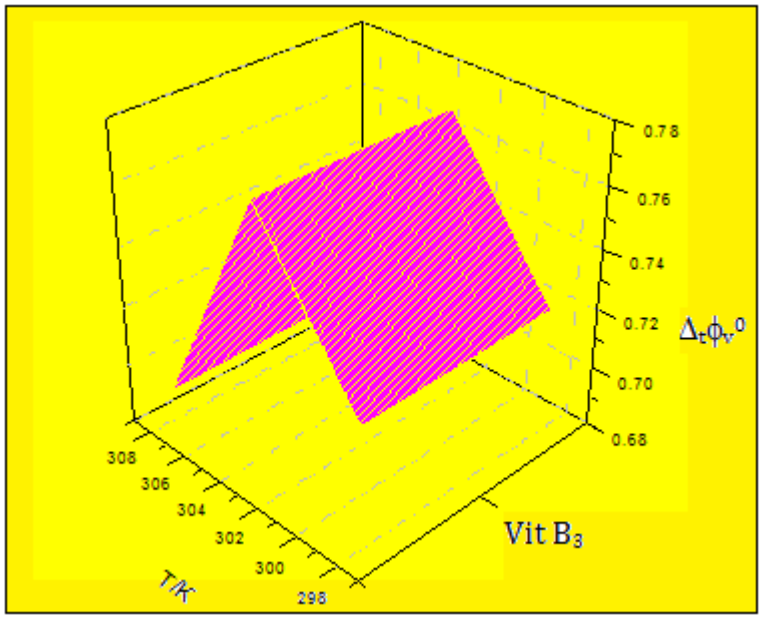

Figure7. Plots of standard partial molar volume of transfer ($\Delta_t\phi^0_V$) for nicotinamide on the molality of beta-cyclodextrin (m βCD) in aqueous solutions at T = (298.15–308.15) K.

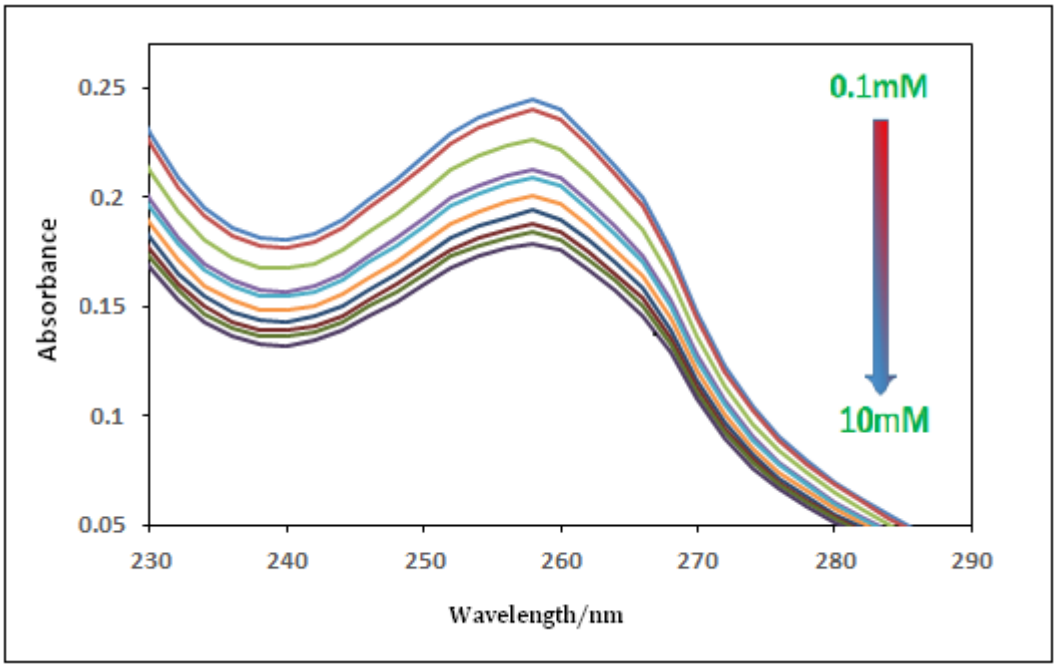

Figure8. Curve of absorbance versus wavelength of nicotinamide (Vit B3) vs. β-CD solution

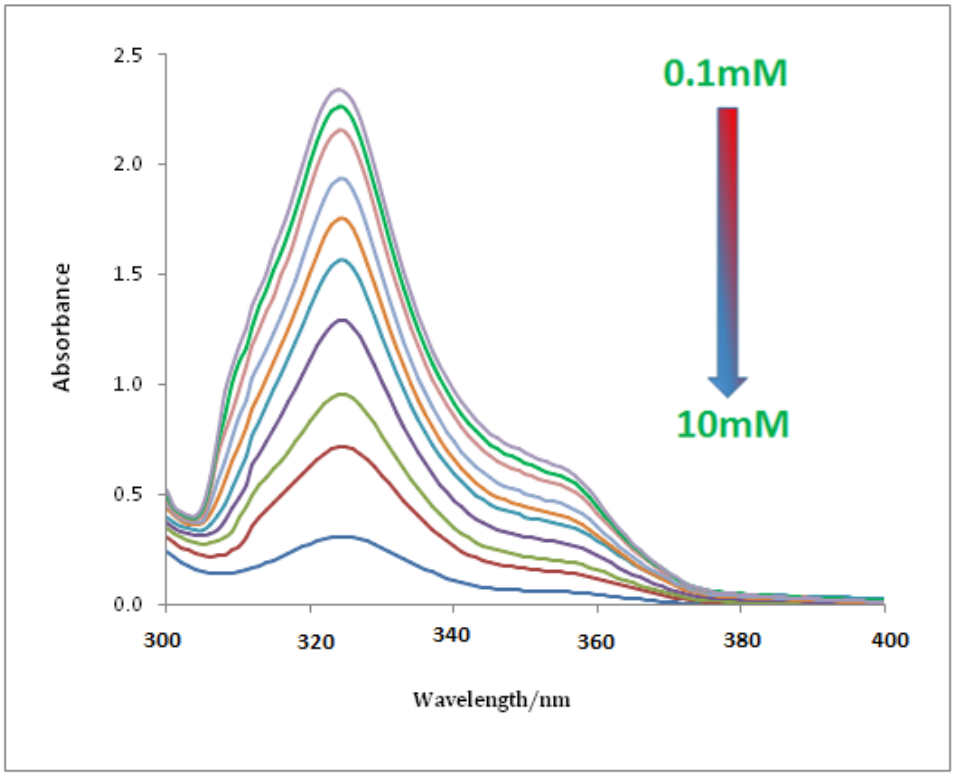

Figure9. Curve of absorbance versus wavelength of pyridoxine (vit B6) vs. β-CD solution

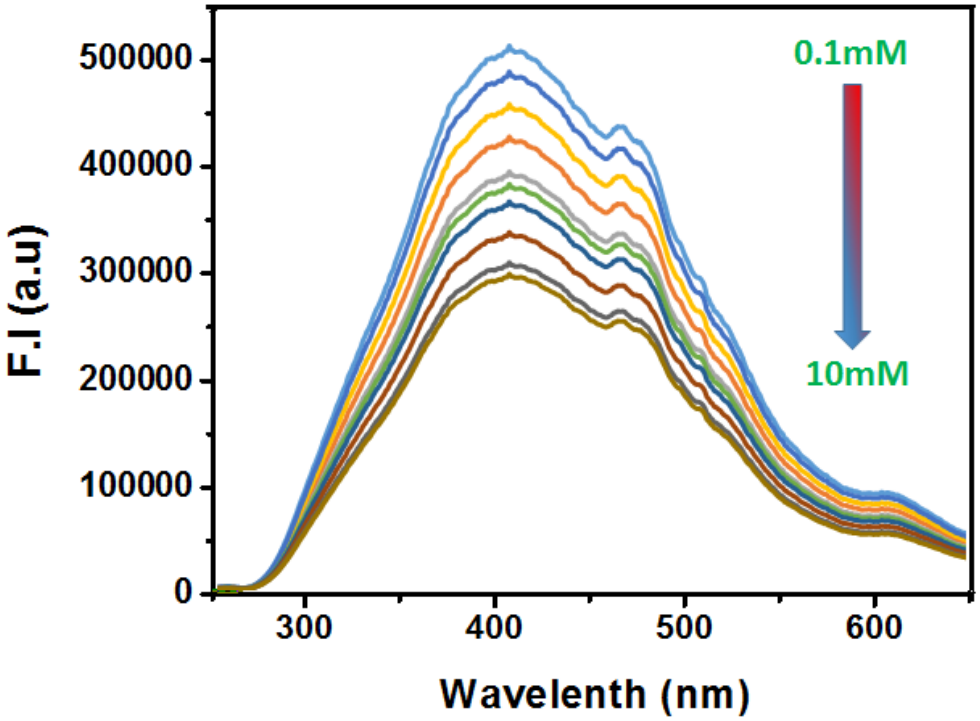

Figure10. Fluorescence emission spectra of aqueous β-CD in the presence of 0.1mM– 1.0 mM of Vit B3 (λex =405 nm, slit =5/5).

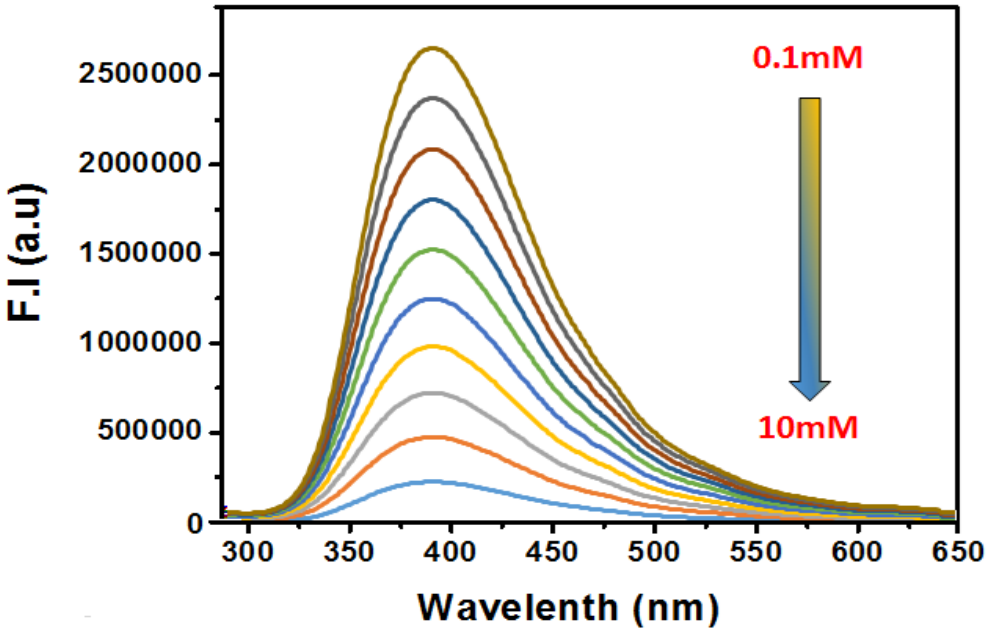

Figure 11. Fluorescence emission spectra of aqueous β-CD in the presence of 0.1mM– 1.0 mM of Vit B6 (λex =258 nm, slit =5/5).

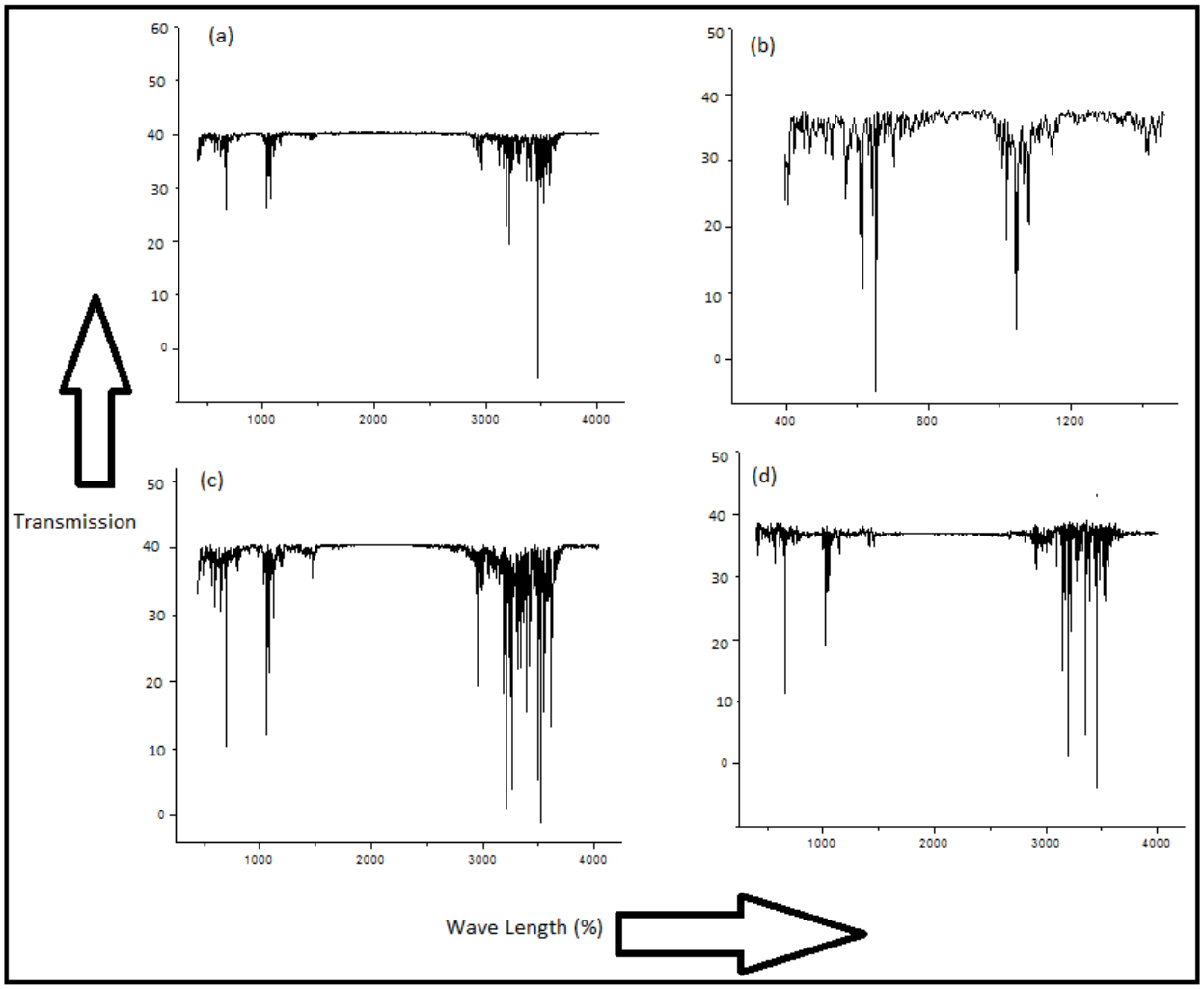

Figure12. FTIR Spectra of (a) nicotinamide, (b) [nicotinamide + β - CD]IC, (c) pyridoxine, (d) [pyridoxine + β - CD] IC.

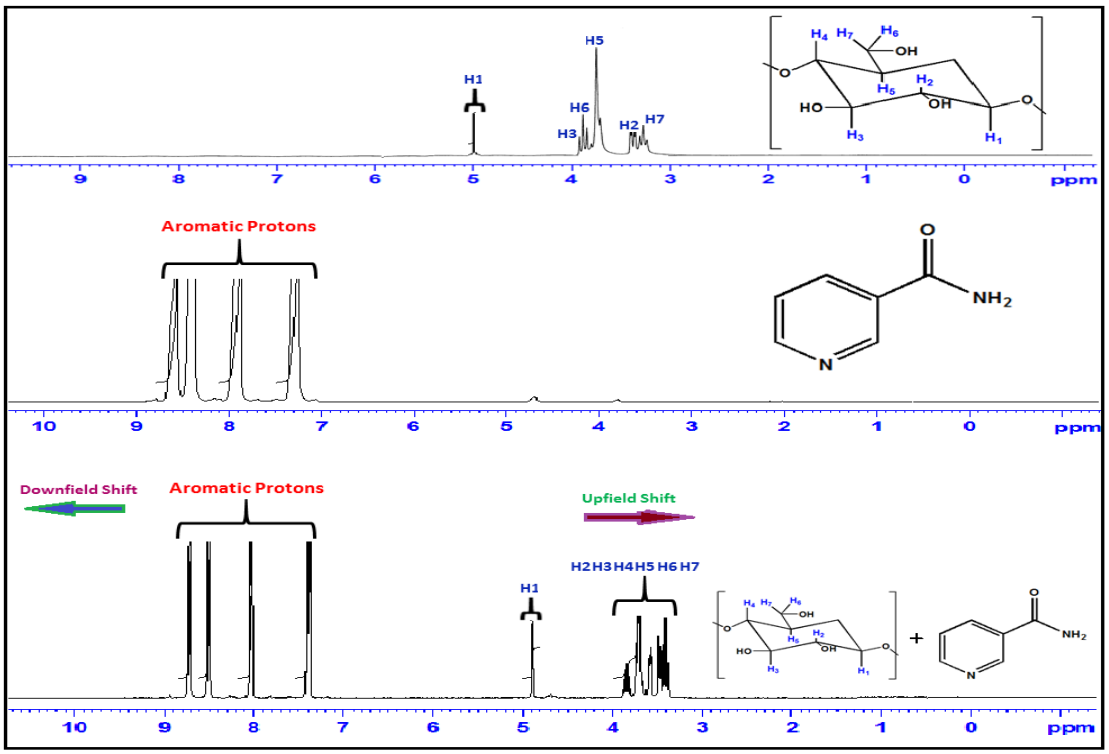

Figure13. 1H – NMR Spectra of [β-CD: Nicotinamide] inclusion complex in D_2O

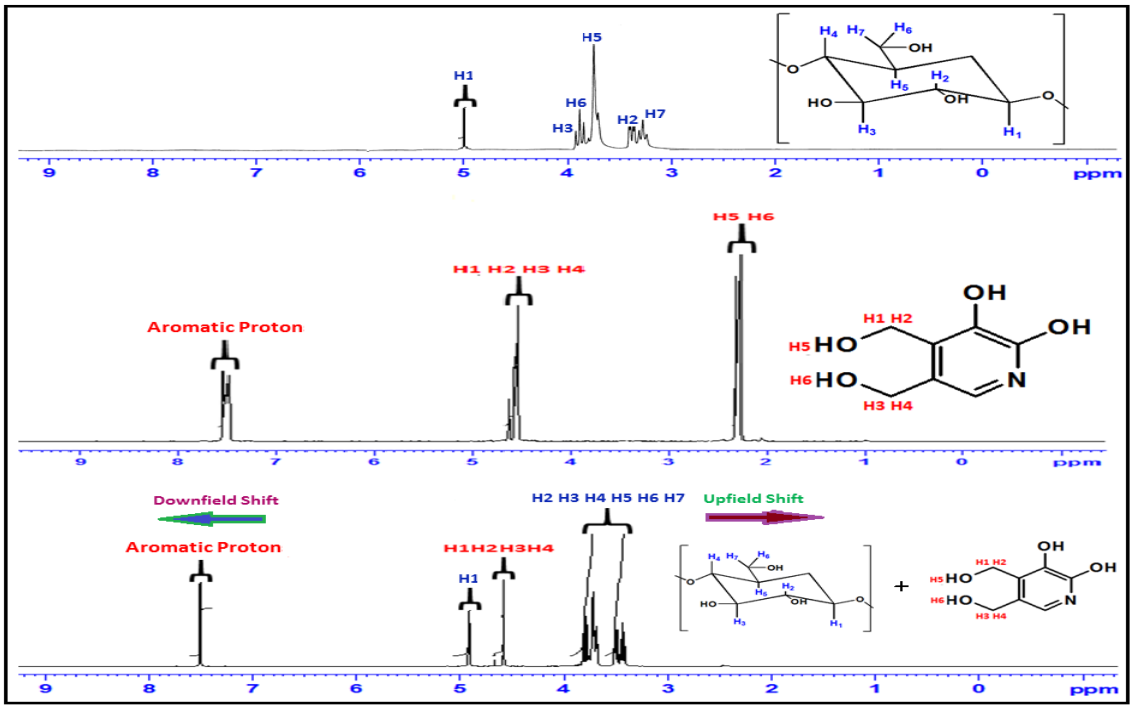

Figure14. 1H –NMR Spectra of [β-CD: Pyridoxine] inclusion complex in D_2O.

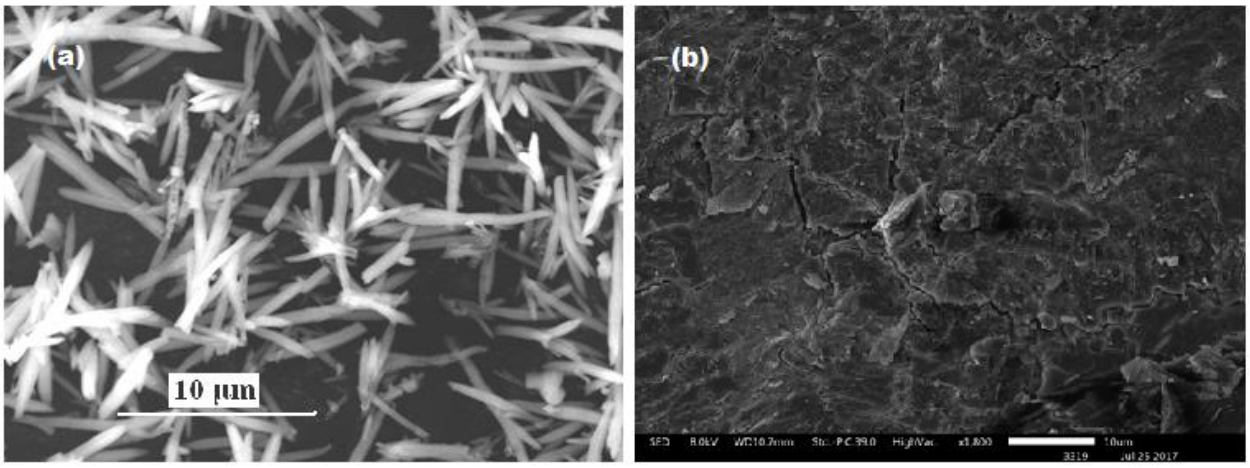

Figure15. SEM images of Vit B3 structures: (a) rod-shaped crystals in the absence of β-CD. With the influence of β-CD (b) uneven micro-clusters formed at pH 7.0.

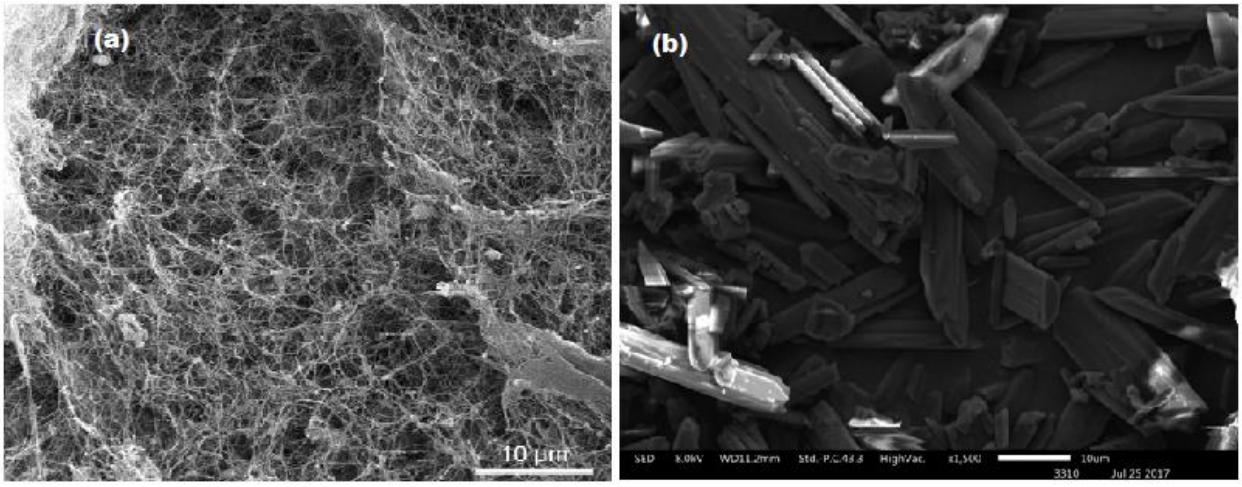

Figure16. SEM images of Vit B6 structures: (a) Mesh is formed in the absence of β-CD. With the influence of β-CD (b) distinct cylindrical rods formed at pH 7.0.

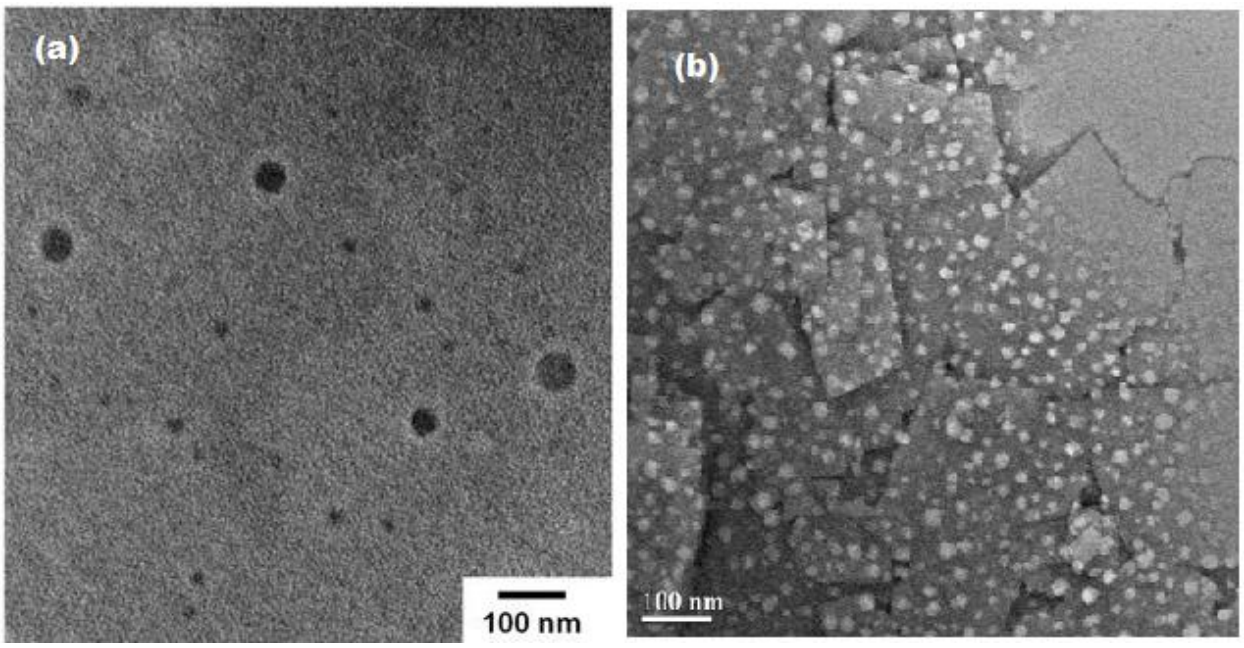

Figure17. TEM images (a) only Vit B3 (b) Vit B3 in presence of β-CD morphological study.

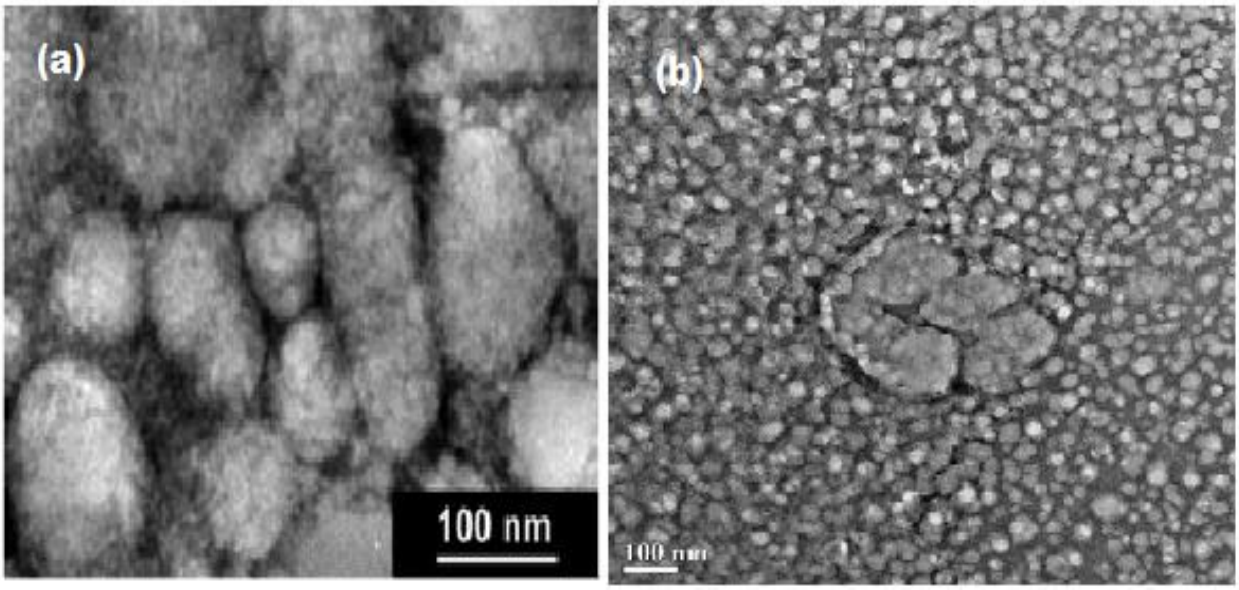

Figure18. TEM images (a) only Vit B6 (b) Vit B6 in presence of β-CD morphological study.

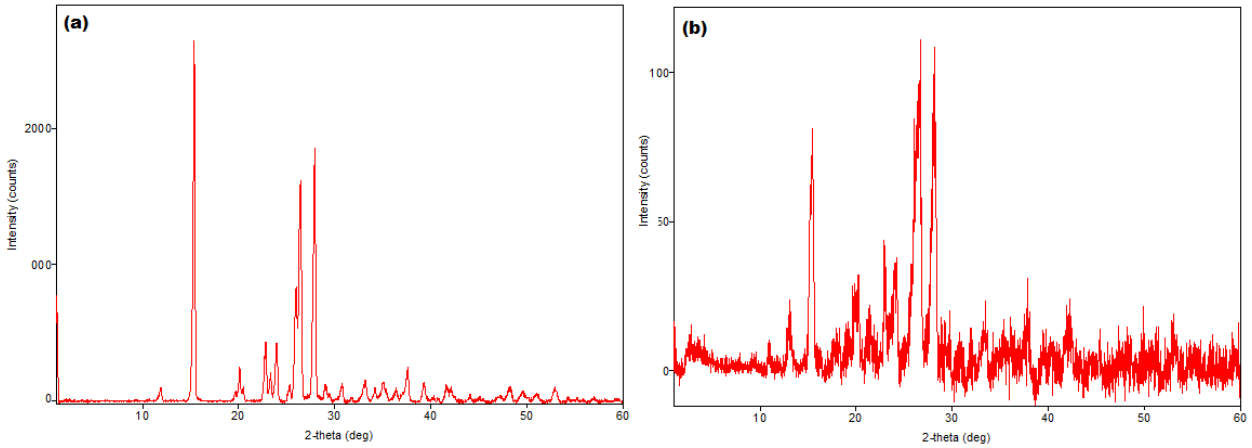

Figure19. Powder X-ray diffraction pattern of (a) Vit B3 and (b) Vit B3: β-CD (1:1M ratio) inclusion complex.

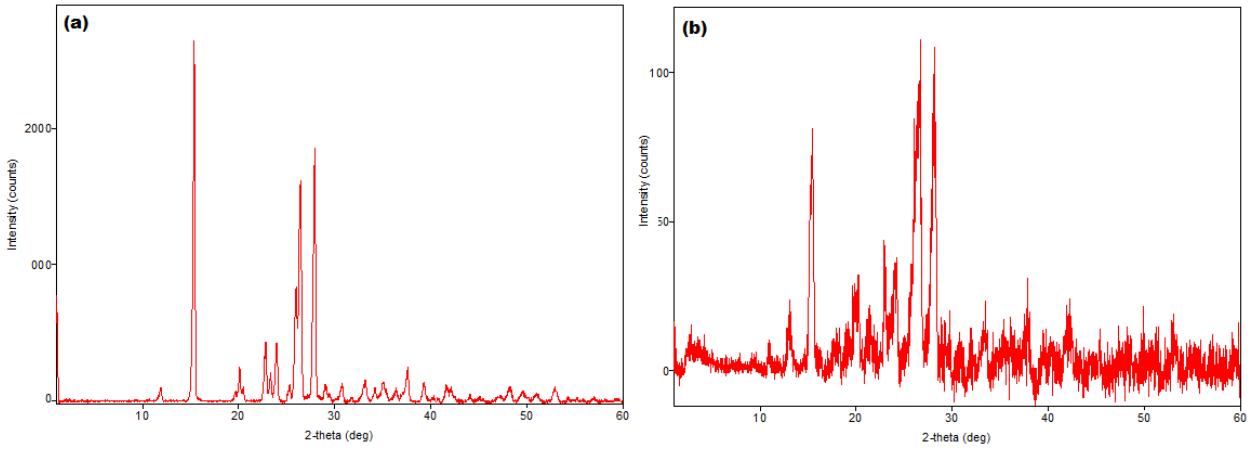

Figure20. Powder X-ray diffraction pattern of (a) Vit B6 and (b) Vit B6: β-CD (1:1 M ratio) inclusion complex.

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