Journal of Inclusion Phenomena and Macrocyclic Chemistry (2005) 51: 27–32

Interactions of Nabumetone with γ-Cyclodextrin Studied by Fluorescence Measurements

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(Received: 24 June 2003; in final form: 11 July 2004)

Key words: γ-cyclodextrin, fluorescence, inclusion complexes, nabumetone

Abstract

The inclusion of the anti-inflammatory drug, *Nabumetone* (NAB), in γ-cyclodextrin (γ-CD) was studied by fluorescence measurements. The emission fluorescence spectrum, of NAB reveals a maximum whose intensity increases with the different γ-CD's growing concentrations. The stoichiometery (1:1) and binding constants of the complexes at 15, 25, 35, and 45 °C were extracted from the analysis of the emission spectra of NAB. The thermodynamic parameters $\Delta H°$ and $\Delta S°$ for the formation of the complex were calculated from the temperature dependence of the binding constants and compared with previous results for similar complexes of NAB with α- and β-CDs. The location of NAB in the complex was determined using the fluorescence quenching method. Our results indicate that NAB is completely penetrated into the cavity of γ-CD.

Introduction

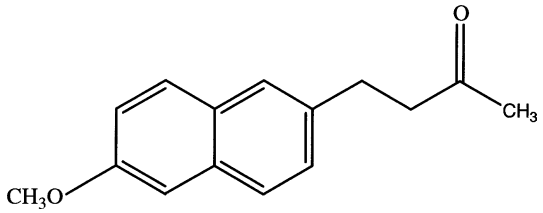
Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucopyranose units linked together via oxygen bridges at the 1 and 4 positions (α-(l,4)-glycoside bonds) [1]. This class of organized media possesses a hydrophilic upper and lower rims lined with hydroxyl groups and a hydrophobic cavity due to C^3H, C^5H, and C^6H hydrogen's and O^4 ether oxygen. This structure gives CDs the ability to extract a variety of organic guest molecules of appropriate size and hydrophobicity from the bulk aqueous solution [2–5]. The most familiar members are α-, β-, and γ-CDs consisting of six, seven, and eight, glucose units, respectively.

Over the last three decades, wide ranges of thermodynamic data concerning the guest–host complexation have been reported. From the large body of these data, the factors contributing to the inclusion complexation were determined [6]. It has been found that the driving forces leading to the inclusion complexation between cyclodextrins and guest molecules are as a result of hydrophobic interactions, hydrogen bonding, van der Waals forces, electrostatic interaction, charge-transfer interaction, the release of high-energy water from the CD cavity and the release of conformational strain upon guest inclusion [7, 8]. Complexation of various guest compounds with CDs generally results in the improvement of some physical properties of the guest molecules, such as stability, bioavailablity, membrane permeability, and solubility [9, 10].

The characteristics of complexation, such as stoichiometery, geometry of the complex, binding constants, and thermodynamic parameters can be evaluated using several techniques. The most common used techniques are ultraviolet–visible absorption spectroscopy [11–16], fluorescence [17–25], nuclear magnetic resonance [11, 26], potentiometrey [11, 27, 28], liquid chromatography [29–33], circular dichroism [17, 18], and X-rays [34].

In luminescence studies, CDs have been employed to enhance fluorescence emission of different luminophors [35–39] and to induce room temperature phosphorescence under appropriate conditions [40–42]. The intensification of luminescent processes of molecules included in the interior of the cavity of the CD is due to the better protection from quenching and other processes occurring in the bulk solvent. CDs have been used to increase the fluorescence intensity of various organic species through a partial encapsulation or total inclusion.

Nabumetone (4-(6-methoxy-2-naphthyl)-butan-2-one; NAB; Scheme 1) is a non-steroidal clinically effective anti-inflammatory drug advocated for use in the symptomatic treatment of rheumatic and inflammatory conditions [43, 44]. Unlike most other drugs of its class NAB is non-acidic and a prodrug. After absorption, NAB forms an active metabolite (6-methoxy-2-naphthylacetic acid), which is a potent inhibitor of prostaglandin synthesis. Three other metabolites: 4-(6-hydroxy-2-naphthyl)-butan-2-one, 4-(6-hydroxy-2-naphthy1)-butan-2-ol and 6-hydroxy-2-naphthylacetic acid have been identified in human urine [45–47]. This drug is poorly soluble in water and exhibits intrinsic fluorescence.



Scheme 1. Chemical structure of nabumetone.

Recently, the inclusion complexes of NAB with β-CD, methylated-β-CD, and hydroxylproply-β-CD were studied by Sanchez *et al.* [48] using fluorescence and molecular mechanics calculations. A 1:1 stoichiometry was obtained for all NAB:β-CDs complexes. The stability constants were calculated at several temperatures. The experiments showed that the formation of NAB:β-CDs had a negative $\Delta H°$ and a positive $\Delta S°$. Molecular mechanics calculations showed that the comp1ex could be formed by penetration of NAB through any of the rims of β-CD. Costa *et al.* [49] used fluorescence and UV–Vis absorption spectroscopy to study inclusion complexes of NAB with β-CD, hydroxylproply-β-CD, and α-CD. A 1:1 stoichiometry was also obtained for all NAB:CDs complexes. The stability constants were calculated at 20 °C. The experiments showed that the formation of NAB:CDs had a negative $\Delta H°$. The formation of NAB:α-CD was accompanied by a decrease in entropy, whereas the formation of NAB:β-CDs were accompanied by an increase in entropy. Conformations of nabumetone in aqueous environments were studied by Costa *et al.* [50] using fluorescence and lifetime measurements. The obtained results showed an evidence for the existence of two conformations of NAB, one with folded structure, and the other is non-interactive extended one. Sanchez *et al.* [51] used X-ray diffractometery, thermal analysis, and infrared spectroscopy to study the interactions of NAB with cyclodextrins in solid state. Also, they used phase-solubility analysis to study the interactions of NAB with cyclodextrins in solution. The experiments showed that the solid dispersions of NAB with γ-CD have a remarkable improvement in the dissolution rate of NAB.

In the present work, spectrofluorimetric measurements were reported to study the complexation of NAB with γ-CD, which contains eight glucose–pyranose units. Fluorescence anisotropy measurements were performed to interpret the sign of $\Delta S°$ upon complexation. The binding constants were determined by spectrofluorimetry. The thermodynamic parameters were calculated by the influence of the temperature on the stability constants.

Experimental

Materials

Nabumetone (NAB) and γ-cyclodextrin, were purchased from Sigma, and used without further purification. NAB and NAB:γ-CDs water solutions for spectrofluorimetry were made using deionized water, which was further fractionally distilled from $KMnO_4$. *m*-Cresol, purchased from Aldrich, was used without further purification. All solutions were stirred at room temperature for 4 h with small magnetic stirrers before measurement. The concentration of NAB was 1×10^{-5} mol/l and held constant in all experiments. The concentrations of γ-CD ranged from 2×10^{-4} to 2×10^{-3} mol/l.

Apparatus

Fluorescence measurements were performed using an SFM 25 Kontron AG spectrofluorimeter equipped with photomultiplier and a double monochromator system and a xenon-high pressure lamp, with a precision of $\lambda \pm 0.1$ nm. Slit widths were 5 nm for excitation and emission. The temperature of the cell housing (1 cm path cells) was controlled with a bath (Techne) equipped with a power head (Tempette TE-8D), covering temperatures from -30 to $+80$ °C with a precision of $\pm$ 0.1 °C. The emission spectra were recorded with excitation, at 330 nm. The anisotropy (r) was obtained from the fluorescence polarization measurements using the single-channel method (*L*-format) described by Lakowicz [52] with 330 and 356 nm for excitation and emission, respectively. Measurements were recorded from 15 to 45 °C at 10 °C intervals.

Methods

Determination of the binding constants

The calculation of binding constants was made using the spectrofluorimetric method. The fluorescence intensity measurements were determined, keeping the concentration of NAB at the same constant value of 1×10^{-5} M, while the concentrations of the γ-CD varied from 2×10^{-4} to 2×10^{-3} M. Each measurement was an average of two experimental values. The excitation and emission wavelengths were 330 and 356 nm, respectively. The binding constants were determined from the fluorimetric data assuming a 1:1 stoichiometry, by using the modified Benesi–Heldbrand equation [53]:

$$\frac{1}{(F - F_0)} = \frac{1}{(F_\infty - F_0)} + \frac{1}{(F_\infty - F_0).K.[\mathrm{CD}]_0} \qquad (1)$$

where F and F_0 are the fluorescence intensities in the presence and in absence of γ-cyclodextrin, respectively. F_∞ is the fluorescence intensity observed when all the NAB molecules were complexed with cyclodextrin. $[\mathrm{CD}]_0$ is the initial concentration of γ-cyclodextrin. K is the binding constant, which was calculated from the intercept and the slope of the straight line obtained by plotting $1/(F\text{-}F_0)$ *versus* $1/[\mathrm{CD}]_0$.

Thermodynamic parameters

The thermodynamic parameters $\Delta H°$ and $\Delta S°$ of the inclusion process were determined from the temperature

dependence of the binding constants, the measurements were recorded from 15 to 45 °C at 10° intervals, using the Van't Hoff equation:

$$\ln K = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \qquad (2)$$

Thus, the thermodynamic parameters $\Delta H°$ and $\Delta S°$ were obtained from the slope and intercept of the Van't Hoff plot of In K *versus* $1/T$.

Results and discussion

Binding constants

The binding constant, K, of the inclusion complex formed between NAB and γ-CD is determined using the fluorimetric method. Figure 1 shows the fluorescence spectra of NAB in aqueous solution at 25 °C for different concentrations of γ-CD. It has been found that the intensity of the fluorescence emission of NAB increases with the concentration of γ-CD. This dependence is believed to be as a result of complex formation between the guest, (NAB) and the host (γ-CD). The fluorescence emission intensity of the inclusion complex of NAB:γ-CD as a function of γ-CD's concentration at different temperature is shown in Figure 2. Short looking at Figure 2, let one conclude that the fluorescence emission intensity increases with the concentration of γ-CD and decreases as the temperature increases.

According to Equation (1), plotting of $1/(F\text{-}F_0)$ *versus* $1/[CD]_0$ at different temperatures result in a straight line (Figure 3). The linearity of the plots confirms the fact that the stoichiometry of the complex is 1:1. The binding constant can be calculated from the intercept and the slope of the straight lines according to Equation (l).

The binding constants, calculated by the fluorimetric method described above, are summarized in Table 1.

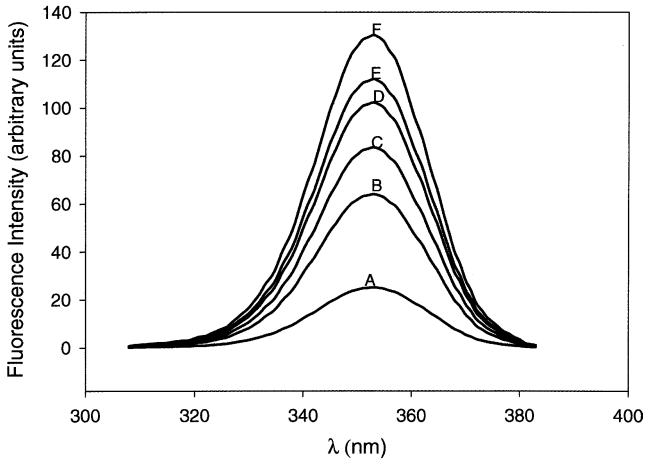


Figure 1. Emission spectra of NAB (1×10^{-5} M) in aqueous solution as a function of γ-cyclodextrin's concentration at 25 °C. [γ-CD] (mM): (A) 0; (B) 0.2; (C) 0.4; (D) 0.7; (E) 1.0; (F) 2.0.

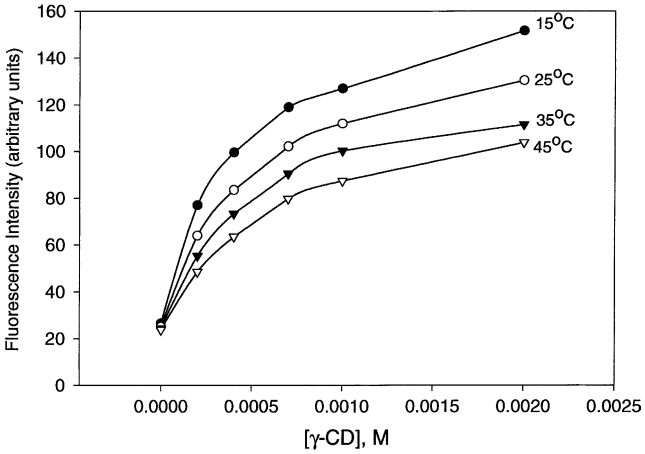


Figure 2. Fluorescence intensity for aqueous solutions of NAB:γ-CD complex at 15, 25, 35, and 45 °C. [NAB] = 1×10^{-5} M.

The values of the binding constants for the NAB:γ-CD complexes are higher than those reported for α-CD [49] and β-CD [48] at all of the experimental temperatures. We attribute this finding to the size effect of the cyclodextrins cavity. Thus, the NAB has higher affinity for γ-CD than for α-CD and β-CD. These results show that the size of the cyclodextrin cavity plays an important role in complex formation. On the basis of size considerations the best fit of NAB can be achieved with γ-CD, whereas in the case of α-CD the NAB is too large to fit nicely into the cavity.

Thermodynamic parameters

The thermodynamic parameters of complexation, enthalpy ($\Delta H°$) and entropy ($\Delta S°$) changes are obtained from the temperature dependence of the

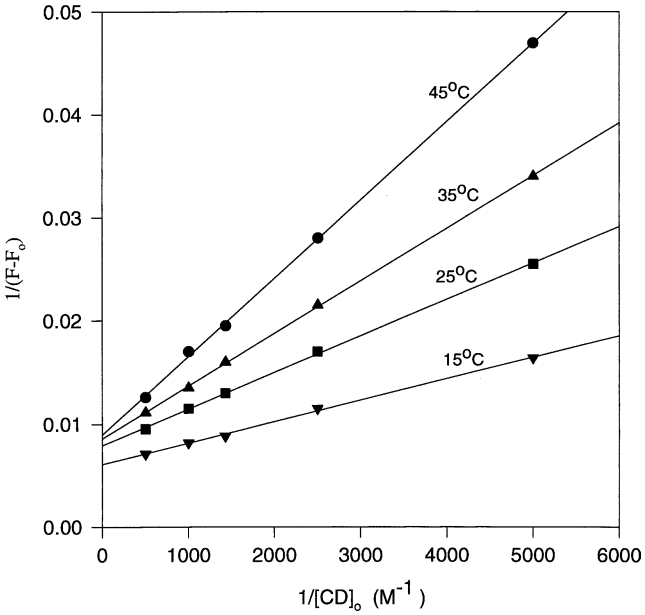


Figure 3. Plot of $1/F\text{-}F_0$ against $1/[CD]_0$ obtained for NAB:γ-CD complex at 15, 25, 35 and 45 °C. [NAB] = 1×10^{-5} M.

Table 1. Binding constants (K) for the inclusion complexes of NAB and γ-CD

Temperature (°C)	$10^{-3}K(M^{-1})$
15	2.9 ± 0.4
25	2.2 ± 0.1
35	1.7 ± 0.1
45	1.2 ± 0.1

Table 2. Thermodynamic parameters ($\Delta H°$ and $\Delta S°$) for the formation of the 1:1 complexes of NAB with three CD hosts

CD	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
α-CD[a]	−25.0 ± 0.3	−54.9 ± 1.2
β-CD[b]	−17.11 ± 0.05	5.31 ± 0.04
γ-CD	−22.90 ± 0.06	12.80 ± 0.05

[a] From [49].
[b] From [48].

binding constants using the classical Van't Hoff plot of In K *versus* $1/T$ (Figure 4), according to Equation (2). The $\Delta H°$ and $\Delta S°$ are calculated and summarized in Table 2. Formation of all three complexes of NAB with α-, β-, and γ-CDs has a negative $\Delta H°$ values. The negative sign of $\Delta H°$ indicates the dissociation of the complex with increasing the temperature. Relative values of $\Delta H°$ are in the order of $\Delta H°_{NAB:\beta\text{-CD}} < \Delta H°_{NAB:\gamma\text{-CD}} < \Delta H°_{NAB:\alpha\text{-CD}}$. The $\Delta S°$ accompanying the formation of NAB:γ-CD is positive and greater than that for the complexation of NAB with β-CD. Thus, NAB:γ-CD and NAB:β-CD complexations are entropically favored. The positive values of $\Delta S°$ are related to the loss of structured water around the guest molecule. In contrast to NAB:γ-CD and NAB:β-CD, the formation of the NAB:α-CD complex is entropically disfavored since $\Delta S°$ is large negative [49].

Fluorescence anisotropy and quenching measurements

Analysis of the fluorescence anisotropy (r) for NAB in the presence of different concentrations of α-, β- and γ-CDs, are summarized in Figure 5. The value of r increases with the concentration of CD due to the presence of the large fraction of complexed NAB, which has a smaller rotational diffusion rate than free NAB. From Figure 5, it has been observed that as temperature increases, the value of r decreases. Such dependence points out the decrease in the fraction of NAB that is complexed and the increase of rotational motion of the probe as the viscosity of the solvent decreases. The values of r for γ-CD and β-CD complexes are in very well agreement with the model that postulates the free rotation of the chromophore inside the cavity. As expected from such model r-value is smaller for γ-CD, which has the larger cavity size. Furthermore, the value of r should be proportional to the size of the NAB:CD complex if it rotates as a rigid unit. In contrast, the smallest complex (NAB:α-CD) has r lying between those of NAB:β-CD and NAB:γ-CD, which may be due to the differences in complexation structure of NAB with the three tested cyclodextrins.

The location of NAB in the complex (i.e. how far NAB is penetrated into the cavity of the cyclodextrin) has been tested using a fluorescence quenching method. In this method, *m*-cresol was used as a quencher. The experiments have been performed on saturated aqueous solution of free NAB, and on an aqueous solution of NAB with excess amount of α-, β- and γ-CDs. Using such procedure of preparation ensures that most of NAB molecules are complexed. In Figure 6, Stern–Volmer plots for the free and complexed NAB are presented. The results show that, at each quencher concentration, quenching of NAB is most effective for free NAB, followed by NAB:α-CD, NAB:β-CD, and least for NAB:γ-CD. Quenching efficiency is interpreted as a measure of the accessibility of the quencher to the chromophore and thus of the location of NAB when

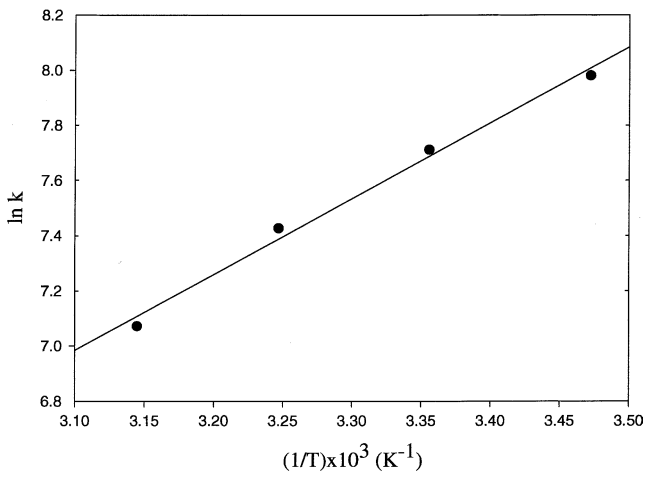


Figure 4. Van't Hoff plot for the formation of NAB:γ-CD complex.

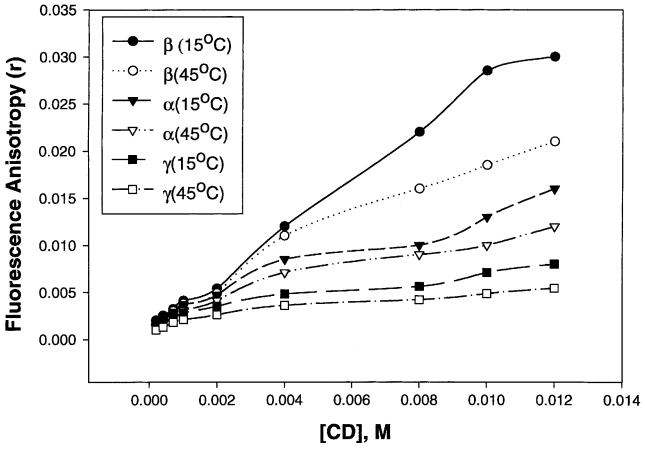


Figure 5. Fluorescence anisotropy as a function of the concentration of cyclodextrins at 15 and 45 °C. γ-CD (squares); α-CD (triangles); β-CD (circles).

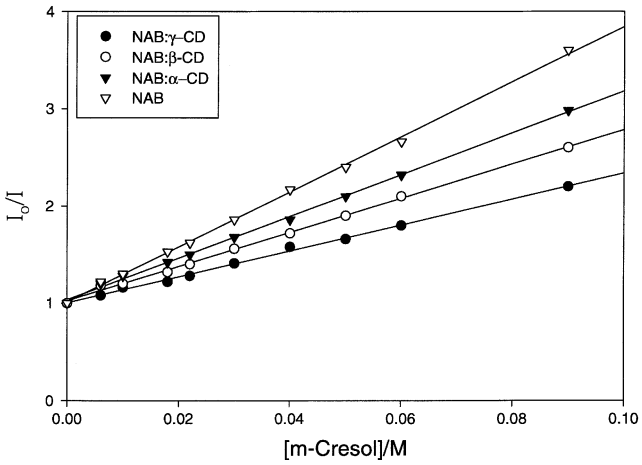
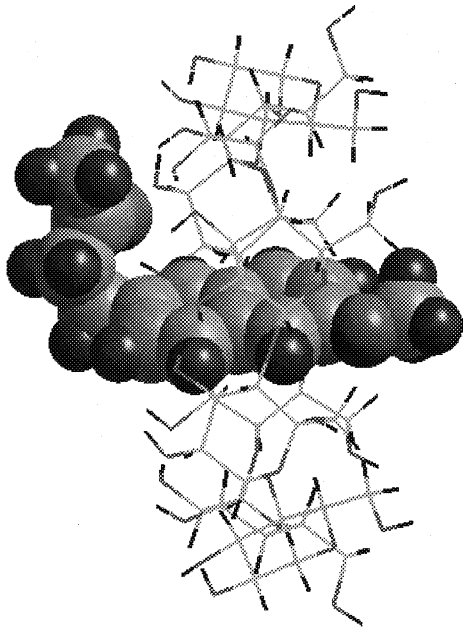


Figure 6. Stern–Volmer plots of the quenching of NAB, NAB:α-CD, NAB:*β*-CD, and NAB:γ-CD by *m*-cresol. $[NAB] = 1 \times 10^{-5}$ M, $[CD] = 2 \times 10^{-3}$M. (I_0/I is the ratio of the unquenched to quenched intensity of luminescence of the maximum emission wavelength).



Scheme 2. Proposed model of the complex between NAB and γ-CD.

forming part of the complex. The results presented in Figure 6, agree with the hypothesis that NAB is slightly penetrated into the cavity of α-CD, while NAB is completely penetrated into the cavities of *β*-CD and γ-CD. The proposed model of the complex between NAB and γ-CD is shown in Scheme 2.

Conclusion

The stoichiometry of NAB:γ-CD is 1:1 and the complexes are thermodynamically stable. The binding constants for the NAB:γ-CD complexes are higher than those obtained for α-CD and *β*-CD at all of the experimental temperatures (15, 25, 35, and 45 °C). The differences in the binding constant are attributed to the size effect of the cyclodextrins cavity. Therefore, the NAB has higher affinity to form complex with γ-CD than with α-CD and *β*-CD. Complexation of NAB with γ-CD is accompanied by a negative $\Delta H°$ values. Compared to other CDs the relative values of $\Delta H°$ is in the order of $\Delta H°_{NAB:\beta\text{-CD}} < \Delta H°_{NAB:\gamma\text{-CD}} < \Delta H°_{NAB:\alpha\text{-CD}}$. The $\Delta S°$ accompanying the complex formation of NAB:γ-CD is positive and greater than the result for the complexation of NAB with *β*-CD. Due to their positive values of $\Delta S°$, NAB:γ-CD and NAB:*β*-CD complexations are entropically favored. The positive values of $\Delta S°$ are attributed to the loss of structured water around the guest molecule. In contrast to NAB:γ-CD and NAB:*β*-CD, formation of the NAB:α-CD complex is entropically disfavored due to the large negative value of $\Delta S°$.

Acknowledgements

Financial support by Jordan university of Science and Technology Research Fund is deeply appreciated.

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