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ORIGINAL CONTRIBUTION

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Behavior of rifampicin in association with β-cyclodextrin in aqueous media: a spectroscopic and conductometric study

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Abstract The behavior of rifampicin (D) with β-cyclodextrin (β-CyD) in aqueous media (W) has been examined by means of UV-vis spectroscopy and conductivity measurements over the temperature range 15–30 °C. The UV-vis study has been used to characterize the systems. The estimated molar absorption coefficient ϵ for D/CyD/W system was 10757 ± 280 M^{-1} cm^{-1} in comparison to the value of 6133 ± 99 M^{-1} cm^{-1} for D/W system. The conductivity was measured (i) as a function of [D] for binary D/W systems, (ii) as a function of [CyD], keeping the concentration of drug constant, for D/CyD/W system, and (iii) as a function of [D] in the presence of a constant cyclodextrin concentration. Two transition points were observed for D/CyD/W system at constant [CyD], which were assigned as cac-1 and cac-2. The stoichiometry of the association was estimated from the conductivity data. This was obtained from [drug] value at which the change in slope of κ occurs. The standard free energy change, ΔG_a^o of aggregation was also calculated from the critical concentration data. An attempt has also been made to estimate the stoichiometry of β-cyclodextrin:rifampicin association.

Keywords Cyclodextrin · Rifampicin · Critical aggregation concentration · Conductivity · UV-vis spectroscopy

Introduction

Drugs are chemical substances which are used for curing diseases and for reducing suffering from pain. Their therapeutic value is due to the physiological effects, which they exert. Water solubility of a drug is the most common limiting factor in the formulation of a pharmaceutical dosage. This limited solubility along with metabolic stability and limited permeability across biological membranes leads to poor bioavailability of drug and in turn affects the drug delivery system. A number of studies have been carried out to overcome this problem, e.g. the dissolution rate can be enhanced either by increasing the surface area of the drug or by complexation approaches [1].

Cyclodextrins (Fig. 1) are cyclic oligomers of α-1,4-linked-D-glucose monomers which form inclusion complexes with several molecules [2, 3, 4, 5]. Various cyclodextrins and their derivatives are proven to be suitable for increasing the solubility and stability of drug [6, 7, 8, 9, 10]. At the same time, some of their derivatives cause sustained release of highly soluble drugs; thus the role of these magical molecules in pharmaceutical chemistry has opened new avenues for research and development. Various research groups have been working on drug:cyclodextrin association.

Uekama [11] has developed 2-hydroxy propyl-β-cyclodextrin (HP-β-CyD) based aqueous formulation of itraconazole (antifungal agent) which was otherwise insoluble in water at physiological pH conditions. The

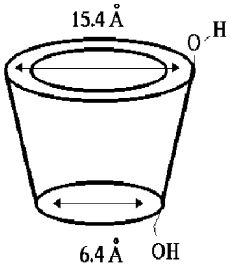

Fig. 1 The cyclodextrin molecule

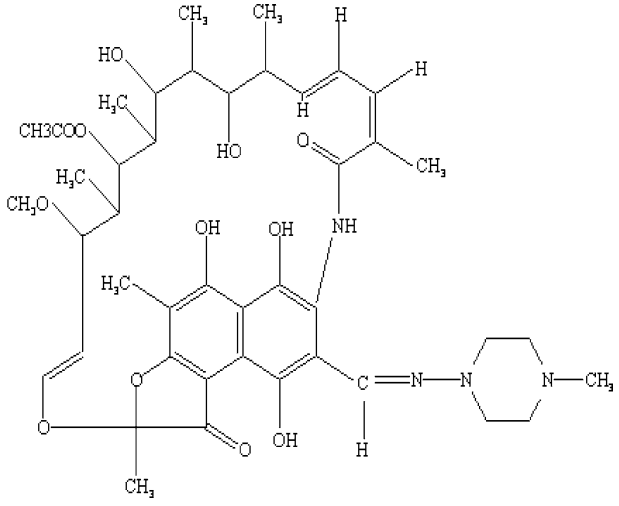

Fig. 2 Structure of the Rifampicin molecule

stoichiometry of the proposed inclusion complex of itraconazole and HP-β-CyD was reported as 1:2. Working on the similar platform, Stefansson and Loftsson [12] used cyclodextrin in formulating eyedrop solutions, which contain corticosteroids such as dexamethasone. They have also studied the effect of water soluble polymer, e.g. hydroxy propyl methylcellulose on the bioavailability of dexamethasone and observed that the co-complex formed between dexamethasone:CyD complex and the polymer enhances the bioavailability of dexamethasone salicylate with β-CyD or 2,6-di-*O*-methyl-β-CyD in aqueous solution. Many works can be found in the literature regarding the low bioavailability of insoluble and unstable drugs highlighting their pharmaceutical aspects. However, it is observed that the physicochemical aspects of Drug:CyD association has not been extensively explored.

Tuberculosis (TB) is highly contagious disease caused by the bacterium called *Mycobacterium tuberculosis*. Rifampicin shown in Fig. 2 is among the most effective drugs against this bacteria. However, it suffer from poor solubility in aqueous media and thus is inconvenient to administrate and in turn causes incomplete cure. Thus there is a need to enhance its solubility by doing desirable manipulations. There are several reports [13, 14, 15] in which efforts have been made in order to enhance the efficiency of delivery and absorption of rifampicin in biological systems. A novel microemulsion formulation [13] capable of delivering rifampicin and isoniazid in combination was formulated despite the difference in solubilities of the compounds. Suarez et al. [14] used a TB-infected guinea pig to screen for targeted delivery to the lungs by nebulization, of either rifampicin alone, rifampicin within poly(lactide-*co*-glycolide) microspheres (R-PLGA) or polymer micro particles alone (PLGA). The study support the potential of R-PLGA delivered to the lungs to treat pulmonary tuberculosis. Recently triglyceride-free pharmaceutical compositions [15] have been formulated for the delivery of various hydrophobic therapeutic agents (including rifampicin). Composition includes a hydrophobic drug and a carrier, where the carrier is formed from a combination of a hydrophilic surfactant and a hydrophobic surfactant. Use of these formulations results in an enhanced rate of absorption of the hydrophobic therapeutic agent. Literature gathered so far urged us to study the behavior of rifampicin in the presence of cyclodextrins and biological surfactants. In the present work, we analyze the behavior of rifampicin in presence of β-CyD, by means of physicochemical studies, namely conductivity measurements and UV-vis spectroscopic measurements. The information obtained from conductivity studies are correlated with the observations of UV-vis studies. The analysis of the results will provide an insight into the dynamics of rifampicin:β-CyD association and serve as the basis for further investigations of such systems.

Experimental

Materials Rifampicin was purchased from Sigma and β-cyclodextrin was purchased from Merck. Both the compounds have purity greater than 98% and were used without further purification. Triply distilled water with conductivity lower than 3 μS/cm was used for the preparation of solutions.

Methods The UV-vis spectra were recorded with HITACHI model 330 spectrophotometer using quartz cells. In the experiments with D/W binary system, the concentration of drug was varied from 0 to 0.08 mmol/l while for theβ-CyD/D/W ternary system firstly drug concentration was kept constant at 0.08 mmol/l and β-CyD concentration was varied from 0 to 0.08 mmol/l. In the other experiment, β-CyD concentration was kept constant at 0.08 mmol/l and drug concentration was varied from 0 to 0.08 mmol/l both in the sample and in the reference cells.

The conductivity of mixtures was measured in a thermostatic glass cell with two platinum electrodes and digital conductivity bridge, Labindia PICO conductivity meter. Electrodes were inserted in a double walled glass cell containing the solution. The glass cell was connected to the thermostat with ± 0.01 K temperature variation. The cell constant of the cell used was 1 cm^{-1} and measurement of conductivity was carried out with an absolute accuracy up to $\pm 3\%$. The conductivity measurements were made at different temperatures, namely 15–30 °C for D/W binary system and for β-CyD/D/W ternary system, as a function of drug concentration keeping β-CyD concentration constant and second as a function of β-CyD concentration with constant drug concentration.

Results and discussion

The UV-vis spectra of aqueous solution of rifampicin is shown in Fig. 3a. The spectra shows four characteristic peaks at 473, 333, 253 and 234 nm, with absorbance value increasing with increase in drug concentration. The data follows Lambert-Beer law (Fig. 4).

Figure 3b depicts the UV-vis spectra of ternary D/CyD/W system. It shows no significant shifts in the characteristic peaks of drug by addition of β-CyD, which indicate the absence of any strong bonding between the drug molecule and β-CyD. The values of absorbance at λ_{473nm} were fitted linearly as a function of drug concentration (Fig. 4). The estimated molar absorption coefficient for D/W system was 6133 ± 99 M^{-1} cm^{-1} whereas for D/CyD/W system it was 10757 ± 280 M^{-1} cm^{-1}. The increase in the magnitude of molar absorption coefficient ϵ indicates the increasing probability of electronic transition in the presence of cyclodextrin.

The ternary D/CyD/W system was further analyzed by varying β-CyD concentration (Fig. 5). After an initial increase in absorbance at low concentration of β-CyD, the absorbance decreases rapidly with increase in β-CyD concentration. A small maximum in the curve points towards the possibility of some association between drug molecule and β-CyD.

Conductivity technique has been found to be highly useful for studying the association behavior of drug systems [16, 17, 18, 19]. In order to gain insight into the type of association taking place between rifampicin (D) and β-CyD, the conductivity method was utilized. For binary D/W systems the conductivity was measured as a function of [D] and for D/CyD/W system as a function of [β-CyD], keeping the concentration of drug constant, or as a function of [D] in the presence of a constant cyclodextrin concentration. The measurements were also carried out at different temperatures, i.e. 15–30 °C. The plots of conductivity κ of rifampicin against drug concentration at different temperatures are illustrated in Fig. 6.

The variation of the conductivity with temperature shows a gradual increase as a function of drug concentration. This may be attributed to an increase

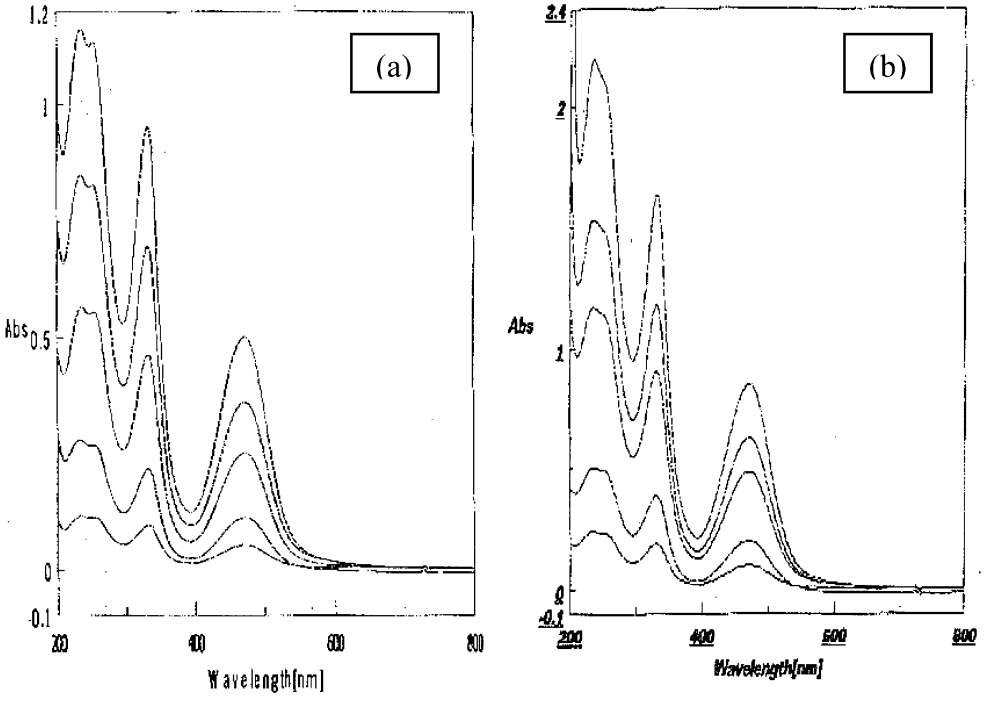

Fig. 3a,b UV-vis spectra of: **a** D/W binary system; **b** ternary D/CyD/W system

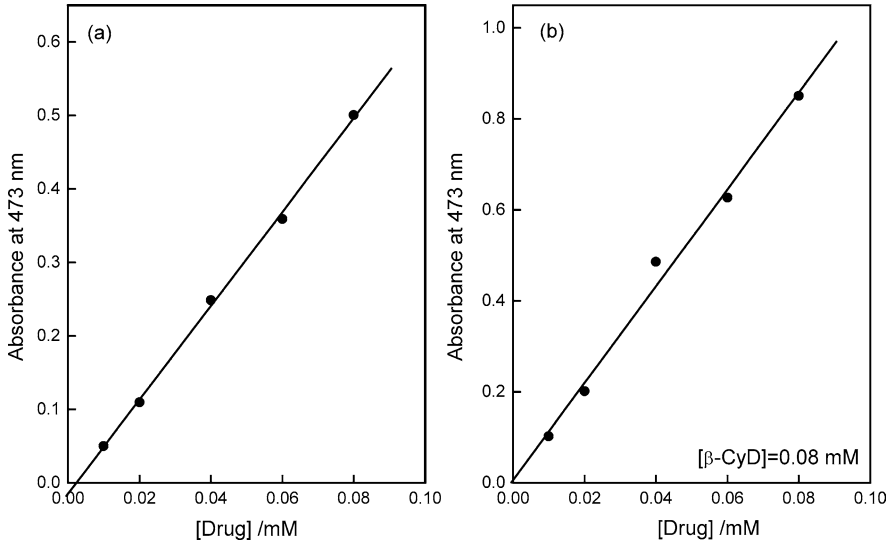

Fig. 4a,b Lambert-Beer linear plots for **a** aqueous solutions of rifampicin at different concentrations; **b** aqueous solutions of rifampicin in presence of 0.08 mM [CyD]

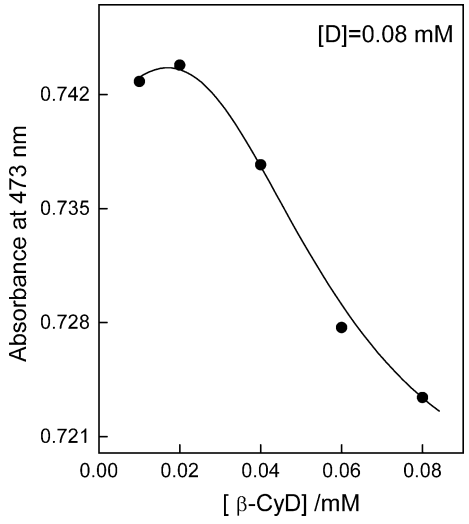

Fig. 5 UV-vis absorption of D/CyD/W system with varying [CyD] having 0.08 mmol/l [D]

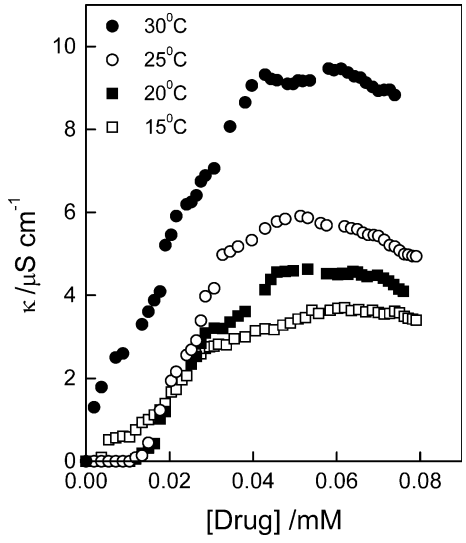

Fig. 6 Conductivity plots for D/W binary system at different temperatures

in the thermal energy of the molecular entities. An abrupt change is also observed around 0.01 mol/l drug concentration in the conductivity at temperatures 15, 20 and 25 °C. However, at 30 °C this change is absent. It may be due to tremendous increase in the mobility of molecules at 30 °C which results in the continuous rise in the conductivity. The critical concentration [20] was determined by the intersection of the two straight lines of the conductivity-concentration plots above and below the change in the slope. One distinct break point is observed at each temperature, which is regarded as *cac* (critical aggregation concentration). The occurrence of this transition point might be due to aggregation of drug molecules. Table 1 lists the *cac* values for D/W system at different temperatures. The standard Gibbs energy change for aggregation of drug is represented by

Table 1 Critical concentration and standard Gibbs energy change of aggregation of rifampicin in aqueous solution at different temperatures

Temperature (°C)	Critical concentration (mmol/l)	ΔG_a^o (kJ/mol)
15	0.060	−32.89
20	0.045	−34.17
25	0.040	−35.11
30	0.039	−35.69

$$\Delta G_a^o = RT \ln X_{cac} \quad (1)$$

where R is the universal gas constant, T is the temperature and X_{cac} is the mole fraction of *cac*.

Figure 7 shows the variation of conductivity as a function of both rifampicin (D) and β-CyD concentration for ternary D/CyD/W system. A sharp change in the slope at various temperatures has been observed which is an indicative of the possibility of association between D and β-CyD.

Moreover, two break points were observed in these plots except at system at 15 °C (Table 2 depicts the *cac* values for this system). The occurrence of these transitions can be visualized in the following manner: initially the system was composed of CyD/W and [D] was varied. As the drug concentration is increased, the drug molecules occupy the available free surface, now the system has free drug molecules along with free CyD molecules which together increases the conductivity of the system. With further increase in drug concentration in the system the availability of free space for drug molecules decreases and thus drug molecules starts moving towards the cavity of CyD by orienting its hydrophobic groups in the cavity. The association is weak but does exist. At comparatively higher drug concentration, drug self-aggregation dominates. Thus the first transition point may be due to CyD:D association and the second is for aggregation of drug molecules. However at low temperature, i.e. 15 °C, due to poor mobility of molecules, the possibility of interaction between D and CyD decreases. Thus no sudden variation in the conductivity at low drug concentration is observed (Fig. 7a). A comparison of cac of the D/W system in Table 1 with the cac-2 of the system D/CyD/W in Table 2 shows that cac (D/W) > cac-2 (D/CyD/W). This suggests that the addition of CyD to the D/W system facilitates the aggregation of drug molecules and thereby a decrease in the cac-2 is observed. The critical concentration with respect to these changes can be confirmed by estimating molar conductivity as shown in Fig. 8.

In Fig. 7b, where drug concentration was kept constant and the CyD concentration was varied, some significant results were observed. The conductivity of the system increases with increase in temperature up to 20 °C; however with further increase in temperature the conductivity starts decreasing and follows the trend $\kappa_{10} < \kappa_{15} < \kappa_{20} > \kappa_{25} > \kappa_{30}$. This indicates that the ternary system is likely to be stable up to 20 °C. Initially the system was composed of drug and water molecules and

Table 2 Critical aggregation and thermodynamic parameters of aggregation of rifampicin in D/CyD/W ternary system with constant [CyD] at different temperatures

Temperature	Critical concentration		ΔG_a^o	
(°C)	(mmol/l)		(kJ/mol)	
	cac-1	cac-2	G-1	G-2
15	–	0.047	–	−33.48
20	0.019	0.034	−36.27	−34.85
25	0.018	0.030	−37.02	−35.76
30	0.017	0.040	−37.79	−35.63

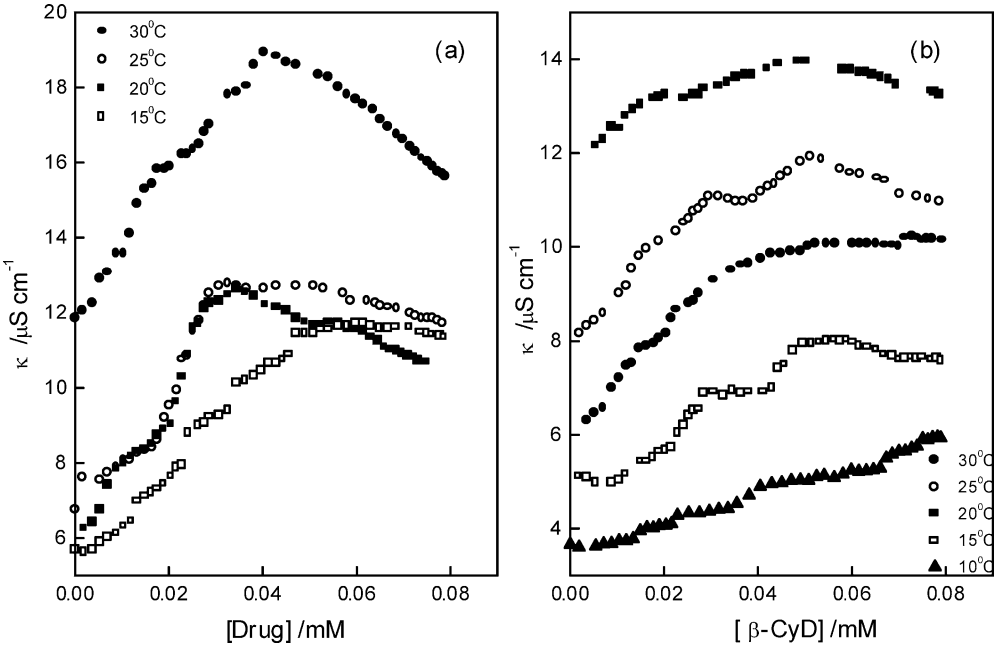

Fig. 7a,b Conductivity κ plots for: **a** D/CyD/W ternary system having 0.08 mmol/l [CyD] with varying [drug] at different temperatures; **b** D/CyD/W ternary system having 0.08 mmol/l [D] with varying [CyD] at different temperatures

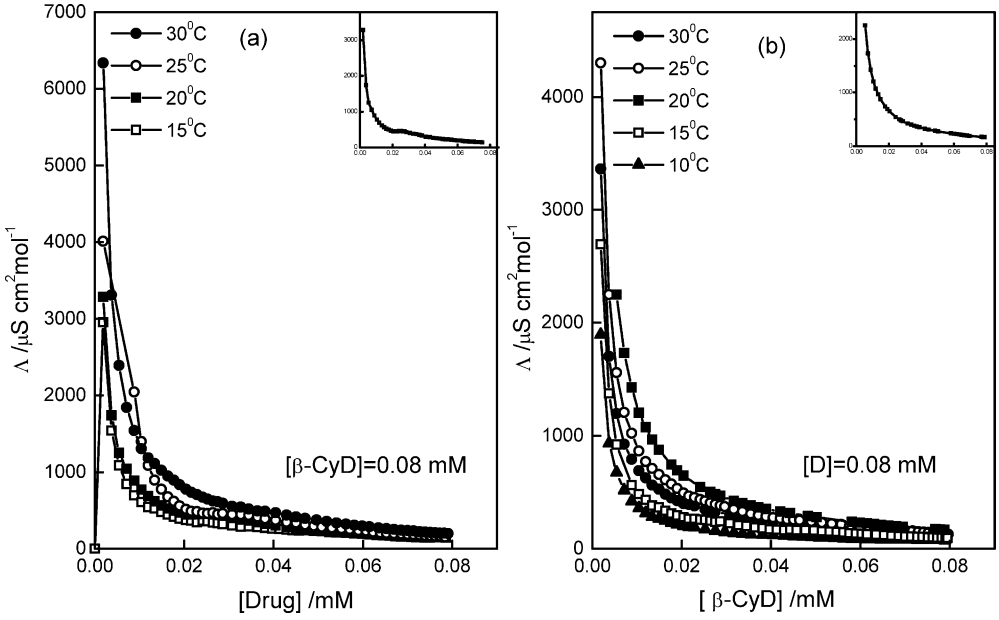

Fig. 8a,b Molar conductance for: **a** D/CyD/W ternary system having 0.08 mmol/l [CyD] with varying [drug] at different temperatures; **b** D/CyD/W ternary system having 0.08 mmol/l [D] with varying [CyD] at different temperatures

cyclodextrin was added. The cyclodextrin molecules occupy the free space available in the system and thus increases the conductivity. With further addition, cyclodextrin molecules tend to move towards the hydrophobic groups of drug and try to encapsulate them, which results in a decrease in conductivity. When the concentration of cyclodextrin is high, it favors self-binding of cyclodextrin molecules. The estimated *cac* and ΔG_a^o for rifampicin in the absence and presence of *β*-CyD are plotted in Fig. 9.

The D/W system both in absence and presence of *β*-CyD when compared indicates the occurrence of weak association between rifampicin and *β*-CyD which is also favored by the decrease in the value of free energy. The stoichiometry [8], of the association is obtained from [drug] value at which change in slope of *κ* occurs. The stoichiometry is the ratio [CyD]/[drug] where, [*β*-CyD] is constant and [drug] is the intersection point of two straight lines at which change in *κ* is observed. On the basis of conductivity data a value of 4 is estimated for stoichiometry at different temperatures.

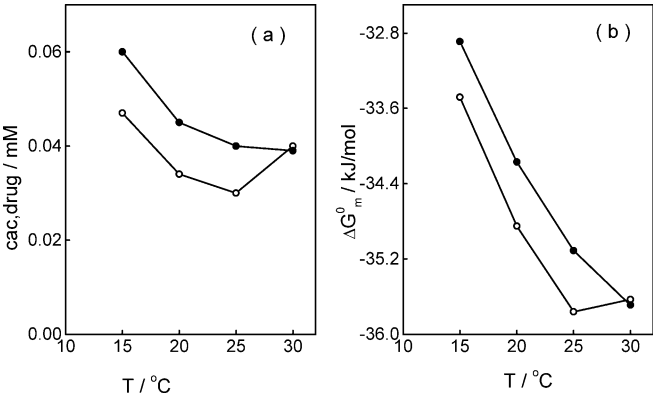

Fig. 9a,b Temperature dependence of: **a** critical concentration cac; **b** change in free energy ΔG_a^o of rifampicin in water (*filled circles*) and in the ternary system of D/CyD/W with constant [CyD] (*open circles*)

Conclusion

Behavior of rifampicin in aqueous media both in the absence and presence of*β*-CyD has been investigated with the help of UV-vis spectroscopy and conductivity measurements over the temperature range 15–30 °C. The increase in the magnitude of molar absorption coefficient ϵ indicates the increasing probability of electronic transition in the presence of cyclodextrin. The conductivity data indicates the occurrence of two break points; the first is due to CyD:D association and the second corresponds to drug aggregation. At 15 °C only one break point is observed which may be due to the poor interaction between D and CyD molecules. It has also been found that critical aggregation concentration (cac) of D/W is greater than the critical aggregation concentration (cac-2) of D/CyD/W. This suggests that the addition of CyD to the D/W system facilitates the aggregation of drug molecules and thereby a decrease in the cac-2 is observed. The results infer the presence of weak association between rifampicin and*β*-CyD in aqueous media which was favored by the decrease in free energy value.

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